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GROWTH CURVES OF INFLUENZA VIRUS BASED ON HEMAGGLUTINATION TITERS IN INDIVIDUAL EMBRYONATED EGGS*

M. W. FREYMANN,† I. TAMM,‡ AND R. H. GREEN

Examination of growth curves, which reflect changes in quantity of the products of viral multiplication over a period of time, offers a promising approach to further understanding of the phenomenon of viral reproduction.

Henle *et al.*[4-6] have described growth curves based upon the titers of pooled allantoic fluids from groups of eggs sacrificed at various intervals of time after inoculation of influenza virus. They have demonstrated the step-like nature of the rises in titer by means of "one-step" curves, which they obtained by utilizing the interfering action of non-infectious virus injected subsequent to the infectious inoculum. Blumenthal *et al.*[1] have recently described a multiple-step growth curve based on the infectivity titers of a series of pooled fluids. Hoyle[7] has studied curves based upon the titers not only of pooled fluids, but also of serial samples from individual eggs. Growth curves in individual eggs, which may be obtained relatively easily by the technique described by Green and Freymann,[2] have an advantage in revealing significant characteristics which may be obscured when pooled fluids are used. Individual curves have been used recently by McClelland and van Rooyen[8] as a means of studying the effect of specific agents on viral multiplication.

The present paper presents growth curves of the PR8 strain of influenza A virus based on serial titrations of hemagglutinin in the allantoic fluids of single infected eggs. These data confirm the findings of previous observers regarding the step-like nature of the curves and regarding an effect of the size of viral inoculum upon the lag period before appearance of detectable hemagglutinin. In addition, there are described a multiple step curve, an effect of age of egg upon the lag period, and some unusual variations of the "normal" curves.

It is realized, of course, that titers of hemagglutinating capacity may not be directly proportional to the amount of infectious virus present in tissues or fluids of the host, and that these curves are but one reflection of the reproductive phenomenon.

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* From the Department of Internal Medicine, Yale University School of Medicine. Aided by a grant from the United States Public Health Service.

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Methods

Embryonated hens' eggs were inoculated with 0.1 cc. of 10-fold dilutions of the PR8 strain of influenza A virus. Dilutions were made in 0.1 molar phosphate buffer having a pH of 7.0. Serial aspirations of 0.2 cc. of allantoic fluid were performed; the interval between the inoculation of virus and the first withdrawal of fluid, and between subsequent withdrawals, varied with the purpose of the experiment. The technique used for aspiration has been described previously.[3] The eggs were incubated at 37° C. Prior to each aspiration, the eggs were candled; those eggs in which no movement could be detected were considered dead and were discarded. Allantoic fluids from embryos which died were cultured aerobically on blood agar plates. Eggs yielding bloody or yolky fluids were discarded. The fluid obtained at each aspiration was titrated for hemagglutinating ability by a modified Salk technique.[11] Allantoic fluid (0.2 cc.) was added to 0.2 cc. of 0.85 per cent saline solution, and serial 2-fold dilutions were made in saline in 0.2 cc. amounts. A single 1.0 cc. pipette was used for each set of dilutions.

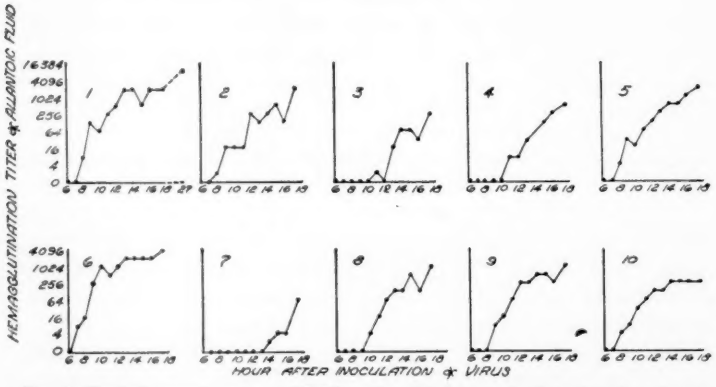

FIG. 1. Typical growth curves of PR8 strain of influenza A virus, based on hourly hemagglutination titrations of allantoic fluids, from individual 12-day eggs inoculated with $10^{0.5}$ EID_{50}. Note individual variations and tendency towards stepwise increases in titer.

To each tube was then added 0.1 cc. of a 1 per cent suspension of chicken red blood cells in saline. Readings were made after one hour at room temperature, and the last tube in which a well-formed (3+) agglutination pattern was visible was taken as the end point. The reciprocal of the original dilution of allantoic fluid in this tube was called the hemagglutination titer.

Results

Contour of typical growth curves. Hourly aspirations of allantoic fluid were performed in several experiments in order to determine the characteristics of the curves. Frequently the smooth rises in titer of hemagglutinin appeared to be interrupted by one or two plateaus or actual drops in titer, which were followed in turn by secondary or tertiary rises in titer. A regular relationship was suggested between the times of inoculation of virus and the times of onset of the secondary and the tertiary rises. Figure 1

shows the results of one experiment, in which a group of 12-day eggs received inocula of a 10^{-1} dilution of virus having an EID_{50} of $10^{-7.5}$. Eggs which survived less than 4 hours after appearance of hemagglutinin were discarded. The primary rises in hemagglutination titer appear to start at widely variable times: between the 6th and 11th hours after inoculation of virus in curves No. 1-6, between the 8th and 14th hours in curves No. 7-9, and between the 7th and 8th hours in curve No. 10. Suggestive secondary rises in titer appear to start between 10 and 13 hours after inoculation in curves No. 1-6 and between 16 and 17.5 hours after inoculation in curves No. 7-9. Tertiary rises in titer appear to start between 15 and 17.5 hours after inoculation in curves No. 1-3. With one exception (egg No. 10), the secondary or tertiary rises appear either between 10 and 13 or between 15 and 17.5 hours after inoculation of virus.

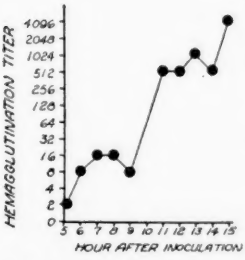

FIG. 2. Multiple step growth curve in a 10-day embryo inoculated with $10^{-8.5}$ EID_{50}.

In another experiment, using the same inoculum, but 10-day eggs instead of 12-day eggs, 14 of 18 eggs produced curves which showed plateaus followed by secondary rises in hemagglutination titer; secondary rises started, on the average, between 9.5 and 10.5 hours after inoculation. In the curves from the remaining 4 eggs no such step phenomenon was demonstrated. In 4 of the 14 curves which showed the usual early plateaus there were also well-defined second plateaus or drops in titer, followed by third and final rapid rises which started between 2 and 6 hours after the second rises. One of these curves is presented in Figure 2.

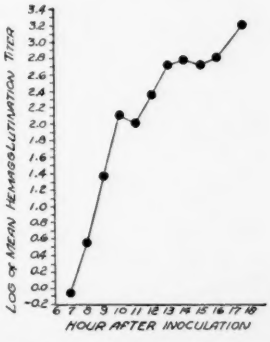

FIG. 3. Composite of curves presented in Figure 1. Each point represents the log of the arithmetic mean of 10 individual titers at a given hour after inoculation. No hemagglutinin was detected in any egg at 6 hours.

In many of the curves described above, a plateau is suggested by a two-fold deviation of titer from the smooth curve. However, with the method used, probably only a four-fold or greater difference between single titers is significant. A composite curve is presented in Figure 3 which is based upon the curves depicted in Figure 1. Each point represents the log of the arithmetic mean of individual titers from 10 eggs. Two plateaus interrupt the smooth rise in titer. These are followed by rapid secondary and tertiary rises which start, respectively, at about 10 and 15 to 16 hours.

An unexpectedly long delay in the appearance of hemagglutinin occurred in a few eggs scattered through several experiments. The curves from these eggs are presented in Figure 4. The rate of multiplication of virus appears slower in these than in other eggs, but data sufficient to establish this are not available. This phenomenon appears unrelated to the size of the inoculum.

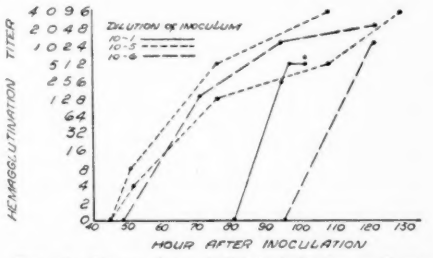

FIG. 4. Curves from individual 10-day embryos demonstrating unusually long delays in appearance of hemagglutinin. Inocula consisted of 0.1 cc. of various dilutions of virus having an EID_{50} of approximately $10^{-7.5}$.

* Test of this fluid for infectivity was positive.

Effect of dose of virus on growth curves. Decreasing the dose of virus prolongs the apparent lag period before the appearance of detectable hemagglutinin in the allantoic fluid. This is illustrated in Table 1 by the results of a typical experiment. In this experiment, fluid was aspirated every two hours, and eggs which survived less than 8 hours after the appearance of hemagglutinin were discarded. The virus used for inoculation was titered simultaneously and found to have an EID_{50} of $10^{-7.5}$. With the maximum dose of virus used in this experiment, $10^{6.5}$ EID_{50}, the time of

TABLE 1

EFFECT OF DOSE OF VIRUS ON INTERVAL BETWEEN INOCULATION AND APPEARANCE OF HEMAGGLUTININ

EID_{50} of virus inoculated		$10^{6.5}$	$10^{5.5}$	$10^{4.5}$	$10^{3.5}$
Hour of detection of hemagglutinin in allantoic fluid:	Average	8.4	12.5	16.3	23.0
	Range	8–10	10–14	14–18	21–25
Number of eggs		10	8	8	6

10-day eggs were inoculated with various amounts of virus. Fluids were aspirated every 2 hours.

appearance of hemagglutinin averaged 8.4 hours. In another experiment, an inoculum containing $10^{8.0}$ EID_{50} of virus was used. In this instance the time of appearance of hemagglutinin in 23 10-day eggs averaged 6.6 hours, with a range of 5 to 8 hours, after inoculation of virus.

Experiments in which small doses of virus were used repeatedly showed more variable results than those in which the doses were larger. This was true with respect both to the interval between inoculation of virus and appearance of hemagglutinin, and to the contour of the growth curve.

Calculations of rates of increase of hemagglutination titers have suggested that the rate may vary with the amount of virus inoculated; however, more data are needed to establish the validity of this observation.

Effect of age of embryos on growth curves. An experiment in which 10-day eggs and 12-day eggs were simultaneously infected with 10^8 EID_{50} per egg revealed that the period between inoculation and appearance of hemagglutinin was approximately 1.5 hours longer in 12-day eggs than in 10-day eggs. In other experiments it appeared that a comparable lag occurred in times of onset of secondary and tertiary rises in titer. The contours of curves obtained in 10 and 12-day eggs were similar.

Comments on technique. An experiment was performed to ascertain that the hemagglutination titer of a small sample of allantoic fluid obtained by needle and syringe was representative of the fluid in the entire sac. Five groups of 10 eggs each were inoculated with a 10^{-2} dilution of virus (about $10^{5.5}$ EID_{50} per egg.) After 13, 24, 48, 72, and 96 hours, two samples of fluid were obtained from each egg in a group, first by aspirating with the needle, then by collecting as much fluid as possible through the air sac with a 10 cc. pipette. The eggs were not chilled, and care was taken to avoid bleeding. There were no significant differences between the hemagglutination titers of the two samples obtained by different methods from the same eggs.

In order to observe the effect on the titer of virus of repeated aspirations of allantoic fluid, some experiments included control groups of 10 eggs which were aspirated for the first and only time at 8, 12, or 18 hours after inoculation of virus. The titers of these control samples did not differ significantly from the titers of samples obtained at the same time from eggs traumatized by 3 to 10 previous withdrawals of fluid.

The mortality rate among embryos subjected to repeated aspirations of fluid varied with the dose of virus, the number of aspirations, and the age of the embryo. As observed by Miller,[9] the mortality was slightly greater when larger doses of virus were used. Regardless of the dose of virus, the frequency of withdrawals of fluid, or the age of the embryo, very few embryos died until after the fourth aspiration. With each subsequent aspiration the mortality rate increased. Twelve-day embryos appeared more hardy than 10-day embryos; about 70 per cent of the former as opposed to about 10 per cent of the latter survived 10 aspirations. The incidence of various technical complications, any one of which disqualified the embryo from an experiment, is indicated in Table 2.

Discussion

Growth curves of influenza virus based on repeated hemagglutination titers in individual eggs demonstrate a rise in titer which may be interrupted by one or two well-defined steps. The times of occurrence of the steps tend to bear a relationship to the time of inoculation of virus and to each other.

It may be postulated that the plateaus represent pauses between "bursts" of release of virus from infected cells; each release causes a sudden rise in titer and is associated with infection of more cells and the onset of another reproductive cycle. Occasional curves show an apparent decrease in the hemagglutination titer during the plateau phase; this may reflect adsorption of the released particles onto new cells.

Measurement of the interval between the terminations of two plateaus (the onsets of secondary and tertiary rises) may provide a means of estimating the length of a reproductive cycle. Application of this measurement to the data presented above indicates a cycle length of 4 to 5 hours. Henle has already suggested that the length of the reproductive cycle of this virus is 5 to 6 hours. His estimate was based on the duration of the interval be-

TABLE 2

COMPLICATIONS ENCOUNTERED DURING THE FIRST 6 ASPIRATIONS IN A GROUP OF 10-DAY EGGS INOCULATED WITH VARIOUS AMOUNTS OF VIRUS

Technical complications	*No. of eggs*
None	63
Death of embryo	20
Bloody fluid aspirated	7
Yolky fluid aspirated	6
Fluid unobtainable	5
Bacterial contamination	4
Total	105

Fluids were aspirated every 2 hours.

tween the inoculation of virus and the appearance of a rise in the level of the infectivity titer of the allantoic fluid.[5]

The interval between inoculation and the rise in the infective property of the fluid is reported to remain constant despite large variations in the size of the infecting dose of virus.[6] The length of the interval between inoculation and the appearance of detectable hemagglutinin, however, bears an inverse relationship to the size of the dose of virus. Larger doses of virus apparently cause earlier accumulation of an amount of hemagglutinin sufficient to be detectable by the Salk test. With the use of doses of virus as large as $10^{9.6}$ EID_{50}, Henle has demonstrated a reduction of this apparent lag period to 4 hours.[4]

Blumenthal, Pinkerton, Greiff, *et al.*[1, 8, 10] have described a curve based on infectivity titers in which suggestive plateaus appear 8 to 10, 14 to 16, and 18 to 22 hours after inoculation. After the 22d hour they have also demonstrated wide fluctuations in the infectivity and hemagglutination titers of the virus and in the oxygen consumption of the embryo. They suggest that

these fluctuations are related to the periodic accumulation and degradation of inactive virus particles which inhibit multiplication of virus, and to variations in the quantity of a viral "toxin" which affects the respiration of the embryo.

The longer lag phase in 12-day eggs than in 10-day eggs inoculated with the same amount of virus may be related to a greater dilution of virus by the greater quantity of fluid in the older eggs.

Examination of curves from individual embryos of the same age inoculated with the same dose of virus reveals that there are often considerable differences in length of the lag period and in contour. It seems likely that these differences are dependent more upon factors inherent in the individual eggs than upon minor variations in techniques, and that they are examples of biological variation in the host, a phenomenon so commonly seen in other laboratory animals. The variations occur more frequently when small inocula are used than when large inocula are used. A possible explanation for the fact that some curves do not show clear-cut evidence of steps is that a number of reproductive cycles overlap, with consequent poor co-ordination of "bursts" of infected cells. The fact that curves without steps are more frequently seen when small inocula are used supports this view. The occasional eggs in which unusually long lag periods occur are particularly interesting. Fluid was aspirated from one infected egg 25 times and contained no demonstrable hemagglutinin until over 97 hours after inoculation.

Summary

1. A selection of growth curves based on serial hemagglutination titers of allantoic fluids from individual eggs infected with the PR8 strain of influenza A virus is presented. A rapid rise in titer appears to be interrupted in many curves by a single plateau, and in occasional curves by two plateaus. The interval between terminations of successive plateaus usually approximates 4 to 5 hours.

2. Decreasing the dose of virus results in a prolongation of the time before appearance of detectable hemagglutinin in the allantoic fluid and in an increase in the variability of this lag period and of the contour of the growth curve.

3. A prolongation of the lag period, without marked change in the contour of the curves, appears when 12-day rather than 10-day eggs are used.

4. Some aspects of the technique used are discussed.

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THE EFFECT OF PHOSPHOLIPIDS ON INHIBITION OF GROWTH AND RESPIRATION IN YEASTS BY SODIUM BENZENE DODECYL SULFONATE*

V. M. CUTTER, JR. AND K. S. WILSON

The germicidal properties of anionic surface-active compounds against various pathogenic microorganisms have been widely reported in recent years.[2] It has been the experience of most workers that inhibition of microbial growth by these compounds is far more effective *in vitro* than *in vivo*. This phenomenon is generally ascribed to adsorption on, or competition between, the surface-active agent and proteins, phospholipids and fatty acids occurring in the host protoplasm, but specific data in support of these contentions are lacking. Because of the importance of a knowledge of the precise mode of action of these compounds to further therapeutic applications, a study of the effects of phospholipids on the fungistatic activity of these compounds was initiated.

It was felt that the phospholipids might be particularly important in this connection because of their presence in the walls and surface membranes of microorganisms. Previously Baker, Harrison, and Miller[1] had suggested that the inhibitory activity of various surface-active compounds might be expressed in part through their capacity to increase the permeability of, or otherwise disrupt, microbial membranes.

The surface-active compound used in this study was sodium benzene dodecyl sulfonate, an anionic substituted alkyl aryl sulfonate. This will be subsequently referred to as SBDS. Activity was measured in terms of the growth and respiration of three yeast species in the presence of SBDS. The organisms tested included *Candida albicans* ♯A-186 isolated from a case of external vaginitis at New Haven Hospital, Connecticut; *Saccharomyces cerevisiae* ♯A-200, a single cell isolate from a gross culture of bakers' yeast; and *Sporobolomyces salmonicolor* ♯13/13-2, a basidiomycetous yeast isolated from tree leaves at New Haven, Connecticut.

The growth experiments were carried out in Ryan flasks of 50 ml. capacity. This type of vessel is an Erlenmeyer flask with a standard Klett-Summerson colorimeter tube attached by its open end at a 45° angle to the lower neck of the flask. This permits full aeration of the flask contents. By tilting the contents of the flask into the tube, successive turbidimetric determinations may be made without the necessity of opening the flasks. Flasks containing 10 ml. of Sabouraud's medium of the following composi-

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tion: 10 grams bacto-peptone, 40 grams dextrose, and 1,000 ml. H_2O were inoculated and shaken continuously at 25° C. on a reciprocating shaker at 140 strokes per minute for a period of 168 hours. Growth was measured in terms of turbidity on a Klett-Summerson colorimeter with all readings corrected for the original inoculum. The data presented are averages of three replicate flasks.

The respiration experiments were carried out using the standard Warburg technique. Washed cells, from 18-hour cultures on Sabouraud's liquid medium, were adjusted to a standard turbidity of 200 Klett units and resuspended in phosphate buffer pH 5 with 2 per cent dextrose added. SBDS and other substances tested were added directly to the phosphate buffer dextrose solution, prior to the final suspension of the yeast cells. The reaction vessels contained 2 ml. of yeast suspension and 0.3 ml. 20 per cent KOH with filter paper fans in the central well. The vessels were shaken at 130 strokes per minute at 30° C. for the duration of the experiment. All experiments on both growth and respiration were carried out at a concentration of 500 ppm. of SBDS and the other substances tested. Respiratory activity was measured in terms of oxygen uptake over a period of 180 minutes. Previous experiments indicated that maximum inhibition of growth and respiration by SBDS under these conditions occurred at pH 5.

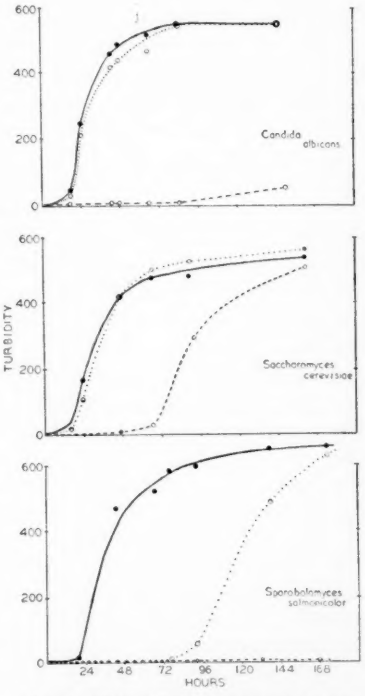

FIG. 1. Effect of SBDS and lecithin upon growth of three yeasts in shake culture. Solid lines: in Sabouraud's medium; dashed lines: plus 500 ppm. SBDS; dotted lines: plus 500 ppm. SBDS and 500 ppm. lecithin.

Figure 1 summarizes the growth of these three organisms in the presence of 500 ppm. of SBDS in liquid Sabouraud's medium in shake culture, and under similar conditions with 500 ppm. egg lecithin plus SBDS. It will be noted that over a period of 168 hours this concentration of SBDS completely inhibits the growth of *Sporobolomyces* and limits growth of *Candida*. *Saccharomyces,* however, adapts rapidly to SBDS after a period of 72 hours. In the presence of lecithin SBDS does not inhibit the growth of

Candida or *Saccharomyces,* while *Sporobolomyces* under these circumstances adapts to the inhibiting concentration of SBDS in 96 hours. The explanation for this increase in the lag phase of *Sporobolomyces* is not clear, but it may relate to the greater sensitivity of this organism to SBDS

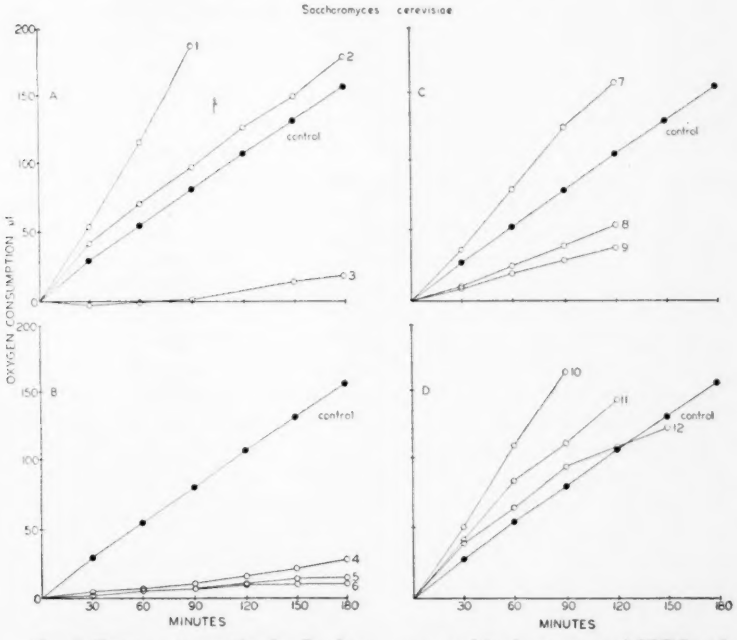

FIG. 2. Oxygen consumption by *Saccharomyces cerevisiae* in presence of SBDS and various constituents of the lecithin molecule. Control: phosphate buffer pH 5 plus 2% dextrose.

A. Curve 1: lecithin; curve 2: lecithin plus SBDS; curve 3: SBDS.

B. Curve 4: Na glycerophosphate plus choline plus SBDS; curve 5: choline plus SBDS; curve 6: Na glycerophosphate plus SBDS.

C. Curve 7: oleic acid plus SBDS; curve 8: stearic acid plus SBDS; curve 9: palmitic acid plus SBDS.

D. Curve 10: Na oleate plus SBDS; curve 11: Na stearate plus SBDS; curve 12: "Tween 40" plus SBDS.

or to the fact that only a few cells remained viable after initial contact with the lecithin SBDS mixture. Further work on this point is in progress. It is apparent, however, that such a phospholipid as egg lecithin is highly effective in overcoming the fungistatic activity of SBDS in the case of organisms such as *Candida* and *Saccharomyces.*

To determine more precisely the actual mechanism of fungistasis by SBDS and its reversal by lecithin, the respiratory behavior of these organ-

isms in the presence of SBDS and lecithin was investigated manometrically. Figure 2A summarizes the oxygen consumption of *Saccharomyces cerevisiae* in the presence of 500 ppm. lecithin (curve 1), SBDS (curve 3), and 500 ppm. of both (curve 2). Lecithin by itself or in combination with SBDS stimulates the oxygen uptake of the cells above that of the glucose, phosphate buffer control. This is undoubtedly due to the liberation of soluble growth-promoting substances during the decomposition of the lecithin. SBDS (curve 3) almost completely inhibits oxygen uptake over a period of three hours. In the presence of equal parts of lecithin (curve 2) this respiratory inhibition of SBDS does not occur.

Since the lecithin molecule contains several fatty acid residues as well as glycerophosphoric acid and choline, these component parts were further tested for their activity in overcoming respiratory inhibition by SBDS. Figure 2B indicates that neither choline (curve 5), sodium glycerophosphate (curve 6), or equal parts of both (curve 4) show any significant reversal of the inhibitory effect of SBDS. Attention was therefore focused upon the fatty acid residues liberated during the decomposition of lecithin. These are dependent upon the particular lecithin, commonly supposed to be mixtures of oleic, stearic, and palmitic acids. Figure 2C shows that oleic acid (curve 7) is as effective as the intact lecithin molecule in overcoming the respiratory inhibition of SBDS. The fact that stearic acid (curve 8) and palmitic acid (curve 9) were less efficient in this connection was attributed to their lesser miscibility in aqueous solutions. This was confirmed by the data presented in Figure 2D in which the more soluble sodium salts of oleic (curve 10) and stearic acids (curve 11) were fully as effective as lecithin. Since sodium palmitate is insoluble in aqueous mixtures, a polyethylene ester of palmitic acid, "Tween 40," which is completely miscible in aqueous media, was substituted. Curve 12 indicates that this ester is almost as effective as sodium oleate and sodium stearate in overcoming the effect of SBDS.

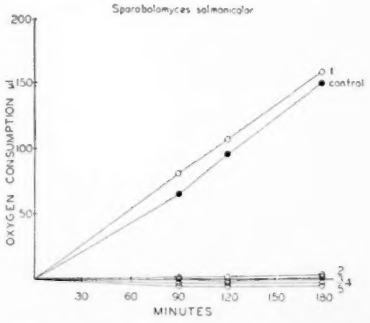

FIG. 3. Oxygen consumption by *Sporobolomyces salmonicolor*.
Control: phosphate buffer pH 5 plus 2% dextrose; curve 1: lecithin; curve 2: lecithin plus SBDS; curve 3: SBDS; curve 4: Na oleate plus SBDS; curve 5: Na stearate plus SBDS.

The respiratory behavior of *Candida albicans* in the experiments described above coincides with that of *Saccharomyces cerevisiae*. Figure 3 shows the oxygen consumption of *Sporobolomyces salmonicolor* in the presence of SBDS (curve 3) and a mixture of SBDS and lecithin (curve

2). Neither lecithin, Na oleate (curve 4), or Na stearate (curve 5) offer any protection against the inhibition by SBDS on this organism. This confirms the data presented in Figure 1 where lecithin did not overcome the inhibition of growth of *Sporobolomyces* for a period of 72 hours.

Since one outstanding difference between strain 13/13-2 of *Sporobolomyces* and the strains of *Candida* and *Saccharomyces* used here lies in the fact that the former possesses high concentrations of several carotenoid pigments, it seemed worthwhile to investigate the possibility that the presence of these pigments might influence the mechanism of inhibition. Consequently an albino mutant of strain 13/13-2 which has no detectable carotenoid pigments was utilized to repeat the experiments. The results with the albino strain (13/13-2-50) coincided exactly with those obtained with the pigmented strain (13/13-2). There seems to be no correlation, therefore, between the presence of carotenoids and inhibition by SBDS.

The data presented indicate that the activity of the phospholipid molecule in preventing the fungistatic effect of SBDS resides in its fatty acid residues. While the results obtained in the growth and respiration experiments with *Candida* and *Saccharomyces* could be explained simply on the basis of chemical combination between fatty acids and SBDS in which the cells play a passive role, the fact that neither phospholipid nor fatty acids overcame inhibition induced by SBDS in *Sporobolomyces* suggests strongly that in this case the protective mechanism is correlated with biological systems. Further work upon the rate of adaptation to, or reversal of, SBDS inhibition by these organisms is in progress.

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THE CARE OF PREMATURE INFANTS ON THE UNIVERSITY SERVICE, GRACE-NEW HAVEN COMMUNITY HOSPITAL*

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It is the purpose of this review to state simply but in detail the present program for the care of the premature infant carried out by the Department of Pediatrics of the Yale University School of Medicine. This program is not remarkable for its uniqueness nor originality, but it is notable that this program has remained with only minor modifications for the past twenty years.[1]

The goal of the program in the care of the premature infant is obvious and all planning is directed toward this aim. It is basically to support the infant who is poorly equipped to exist in the normal environment until he is adaptive enough to be cared for by his parents. By definition, here, the premature infant is considered to be any live-born infant weighing 2,500 grams or less. Time of gestation, maturity level, body length, sex, and race are considered as aids in prognosis but are not utilized in the determination of prematurity.

In general, the care of the premature may be divided into three somewhat overlapping periods. First is the emergency phase which lasts from birth until about seven days. During this period of time the major problems of the premature are with respiration and maintenance of body temperature. All efforts are directed toward providing an adequate supply of oxygen and a rigidly controlled environmental temperature. The second period in the management of the premature infant may be called the alimentary phase which lasts from about the third day to the twentieth day of life. During this time, feeding is initiated and developed until consistent weight gain occurs. When the infant no longer has respiratory difficulty and the ingestion of adequate food is attained, he may be considered to be in the growth phase of premature care. During this period enough food for adequate weight gain is given and an environment relatively free of potential infectious agents is provided. At the end of this time he is considered mature enough to adjust to an adequate home situation. The care during each of these phases is fairly rigidly planned and executed.

Emergency phase. All newborn babies weighing under 2,450 grams are transferred to the premature unit which is under strict isolation precautions and in which individual gown technique without masks is used. The occasional vigorous baby whose mother is particularly desirous of breast feeding him may be cared for in the Rooming-in Unit on the obstetrical floor. The

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smaller, more feeble infants (less than 2,100 grams) are placed in an Armstrong bed in which oxygen up to concentrations of 65 per cent can be supplied and in which a satisfactory regulation of environmental temperature can be maintained. The optimal environmental temperature for these infants has not yet been established, but temperatures under 80° F. and over 88° F. are not considered advisable. A relative humidity of approximately 60 per cent is maintained. The inhalation of 95 per cent oxygen and 5 per cent carbon dioxide is carried out for five minutes every hour for twelve hours and then as needed thereafter in an attempt to expand atelectatic pulmonary areas. No effort is made physically to stimulate the infant to cry, and handling, weighing, suctioning, or examination of a feeble infant are done as little as possible, but voluntary movements are unrestrained. A special nurse, however, is provided for their constant observation for at least twelve hours after birth. Chemical stimulants are used only as emergency measures—caffeine and epinephrine being given intramuscularly or intravenously for respiratory or cardiovascular failure in small doses (0.1 to 0.3 cc.).

Although the exact status of hypoprothrombinemia in the production of hemorrhagic diathesis in the newborn is unsettled, vitamin K (5 mg. intramuscularly) is administered to every infant on admission to the premature unit.

No oral feedings are given for the first twenty-four to forty-eight hours of life, and only then if there is no respiratory distress. In this way aspiration with resulting obstruction and pneumonitis is avoided. With careful observation by nurses and with almost constant attendance by the house staff it is possible to save certain infants who would have died from cardio-respiratory failure.

Alimentary phase. When the premature infant is no longer in respiratory distress, oral feedings are begun. These feedings are designed initially to supply only a small portion of the total caloric turnover of the infant; each day a small amount is added. During the first day of feeding only 30 Calories per kilogram of body weight are given by mouth, and the rest of the day's fluids up to 75 cc. per kilogram of body weight is given parenterally. On the next day, milk mixture (55 Calories per kilogram) is given as well as enough parenteral fluid by clysis to assure an intake of 110 cc. per kilogram. By the third day of feeding 75 Calories per kilogram may be given by mouth and only 20 cc. per kilogram given by clysis. On the fourth day in this routine the premature infant receives no more parenteral fluids and is fed adequate calories (90 Calories per kilogram) to meet the average energy requirements for maintenance. From that time on, small increments to the feedings are made gradually every two to three days until approximately the eighteenth day of life, when the infant is taking by mouth enough calories to effect a small consistent weight gain (120 to 130 Calories per kilogram of body weight per day).

The method of feeding during this time is almost always bottle feeding. In the case of feeble infants dropper feeding is attempted, but if slow and accompanied by respiratory distress, it is replaced by gavage feeding carried out by a graduate nurse. Breck feeders are seldom used. The time interval between feedings is approximately three hours, and self demand feeding has not yet been attempted.

The feeding mixture used is that which was recommended by the senior author over twenty years ago. It is a cow's milk mixture containing 2 per cent fat to which is added 10 per cent sugar by weight. This mixture, at present, is diluted with water so that the caloric density is 0.7 Calories per cc. By the use of such a mixture an adequate water intake is obtained (140 cc. per 100 Calories of food). In this milk mixture approximately 65 per cent of the calories are in the form of carbohydrate, 20 per cent are as fat, and 15 per cent are in the form of protein. To each day's feeding mixture over 100 cc., there is added 0.6 cc. of a water-miscible poly-vitamin preparation. This supplement provides 1.0 mg. of thiamine, 0.4 mg. of riboflavin, 5.0 mg. of niacin, 50 mg. of ascorbic acid, 5,000 units of vitamin A, and 1,000 units of vitamin D.

Crude liver extract—0.5 cc. per kilogram of body weight—is given intramuscularly beginning twelve hours after birth and every five days thereafter until discharge. For the period January 1950 to January 1951, vitamin E has been given in 50 mg. doses three times daily as experimental prophylaxis for retrolental fibroplasia. Vitamin E has not prevented the development of this disease.

The parenteral fluids which are administered by clysis consist of a mixture of equal parts of 5 per cent glucose and Darrow's interstitial salt solution. This salt solution has the approximate composition of an ultrafiltrate of plasma and is similar to Hartmann's lactate Ringer's solution. Hyaluronidase is used to facilitate absorption of these solutions.

Growth phase. With feeding of adequate nutriments for growth and weight gain the premature infant is transferred stepwise to a less rigidly controlled environment. All prematures are removed from the temperature controlled Armstrong beds when the body weight reaches 2,000 grams if there is no respiratory distress and body temperature has stabilized. On attaining the weight of 2,100 grams the infant is transferred to the graduate premature nursery where there is less rigid supervision.

An increase in food intake is made with each significant increment in body weight so that the caloric intake is maintained at 130 Calories per kilogram. The indications for blood transfusion and chemotherapy are in general the same as for other infants.

When the body weight of the baby approaches 2,500 grams, preparations for discharge are made. A complete blood count and urinalysis are obtained, and the time interval between feedings is lengthened if possible to four hours. An appraisal of the home situation is obtained from the Visiting

Nurse Association. If this report is satisfactory, the infant is discharged to the care of the parents and to the medical supervision of a private physician or a child health conference.

Educational care

Not only the physical development of the premature infant must be considered, but also the emotional responses of his parents. This phase in the care of the premature has been greatly improved recently under the guidance of Dr. Sylvia N. Preston. At all times the house staff is available to talk with parents about their infant. However, in addition, on the infant's thirteenth day of life a definite appointment is made for an interview with the parents to discuss with the intern any problems which the parents may have concerning their new baby. At that time a social service worker may be available for social or financial planning and some general prognosis can be given.

Each month a small informal parents' group is held in the evening by one of the pediatric resident staff. In this meeting, concepts regarding the premature, his mental development, his feeding, his acceptance by the other children, etc., can be clarified, while positive suggestions for his care can be made. Parents who are shy and reluctant to question the physician when alone may be drawn out by the questions of other parents.

Prior to discharge of the infant, mothers are encouraged to come to the graduate premature unit as often as possible to feed their babies with the help of a pediatric nurse if needed. In this way parents as well as infants are prepared for discharge.

Follow-up care. The adequate care of the premature, like that of the full-term infant, does not cease upon discharge from the hospital. Home visits to ward patients are made by the pediatric nursery intern within the first week after discharge. This service provides guidance to the mother in her own environment and may prevent some serious error in the home care of the infant.

Two to three weeks after discharge all premature babies are seen in an afternoon follow-up clinic by the intern who was responsible for the baby while in the hospital. At that time a thorough physical examination is performed and laboratory procedures are carried out as indicated. This clinic visit has not only service value to the patient, but also educational value to the intern.

In addition to the routine follow-up noted above, a careful, systematic study of the eyes of all premature infants has been carried out here for the past two years. As soon as an infant can withstand the stress of ophthalmoscopic examination a thorough search for early evidence of retrolental fibroplasia or other ocular disease is carried out. At weekly intervals thereafter, each infant is re-examined until the time of discharge. After discharge from the hospital further examinations are performed every two weeks

until approximately four months of age at the Eye Clinic for Prematures. In this way evidence is being acquired regarding the course and pathogenesis of retrolental fibroplasia which may be useful in an experimental approach to the etiology of this disease.

Summary

The general principles for the care of the premature infant on the University Service, Grace-New Haven Community Hospital have been given. The physical care of the infant and the educational program for the parents are described. The practical outline for the management of the premature is presented below.

Revised rules for the care of the premature

1. All newborn babies under 2,450 gm. to be transferred to Pediatric Service (Fitkin 3).
2. All newborn babies under 2,200 gm. to be put in premature room.
3. All newborn babies under 2,100 gm. to be put in Armstrong bed.
4. All prematures to be removed from Armstrong bed as soon as weight has increased to beyond 2,000 gm.
5. All prematures to be removed from premature room as soon as weight has increased to or beyond 2,100 gm.
6. Routine orders:
   a. On D. L.
   b. Nothing p.o. for first 24 hours of life.
   c. Suction p.r.n.
   d. 5% CO_2—95% O_2 q.1h. for first 12 hours; p.r.n. thereafter.
   e. In case of death, obstetrical assistant resident to be notified at the same time that report operator is notified.
7. All prematures to be given parenteral vitamin K (5 mg.) on admission. Fresh blood or preferably fresh plasma to be given at once if there are any manifestations of hemorrhagic disease.
8. Any newborn weighing less than 2,200 gm. to be specialed for the first twelve hours of life.
9. Prematures are *not* to be stimulated to cry.
10. Regime for second 24 hours of life:
    a. Total fluids to be 75 cc. per kg. of which 40 cc. per kg. is to be parenteral and in the form of one-half 5% glucose, one-half Darrow's solution, and 30 Calories per kg. as oral fluid.
    b. Very careful preparation of skin with alcohol prior to giving clysis should be done in all cases.
11. Third 24 hours of life:
    a. 40 cc. per kg. by clysis as above.
    b. 55 Calories per kg. by mouth (71.5 cc.): Total = 110 cc./kg.
12. Fourth 24 hours of life:
    a. 20 cc. per kg. by clysis.
    b. 75 Calories per kg. by mouth (97 cc.): Total = 117 cc./kg.

13. Fifth 24 hours:
    a. No clysis.
    b. 90 Calories per kg. by mouth.
14. Sixth 24 hours: Same as the fifth.
15. Seventh 24 hours:
    a. Increase to 100 Calories per kg.
    b. No clysis.
16. Eighth 24 hours, ninth 24 hours, and tenth 24 hours: Same as seventh.
17. Eleventh 24 hours:
    110 Calories per kg. by mouth.
18. Twelfth and thirteenth 24 hours: No change.
    Make appointment with family for conference with intern.
19. Fourteenth 24 hours: Increase to 120 Calories per kg.
20. Fifteenth, sixteenth, and seventeenth 24 hours: No change.
21. Eighteenth 24 hours:
    a. Maintain caloric intake between 120 and 130 Calories per kg. daily.
    b. Treatment to remain same throughout remainder of course.
22. Kahn, urine, and C. B. C. to be done prior to discharge by intern.
23. To each formula, add the following daily vitamin supplements:
    0.6 cc. of Vi-Penta (10 gtts.) q. d. which contains:

Thiamin	1.0 mg.
Riboflavin	0.4 mg.
Nicotinic acid	5.0 mg.
Ascorbic acid	50.0 mg.

    Vitamin A—5,000 U.
    Vitamin D—1,000 U.
24. Beginning 12 hours after birth and continuing q. 5 days give the following intramuscularly: liver extract (crude) 0.5 cc./kilogram.
25. Refer all prematures to the Premature Eye Clinic 2 weeks after discharge.

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STUDIES ON THE INACTIVATION OF INFLUENZA VIRUS

Comparison of the Effects of *p*-Benzoquinone and Various Inorganic Oxidizing Agents*

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Infection of susceptible host tissue with influenza virus appears to be dependent upon several factors. In part, these are related to the host cell or receptor, to the chemical environment of the cell-virus reaction, and to the integrity of the virus particle itself. Modification of infectivity may be achieved by altering any one of these factors in such a manner that the virus particle is prevented from entering the host cell or will not multiply after invasion. In addition to specific antibody, the presence in the reacting medium of any substance that competes with host cell receptors decreases the infectivity of the virus, presumably by interfering with adsorption.[4] A state of relative refractiveness to infection may be produced by prior exposure of animals to the "enzymic" action of cholera vibrio filtrates,[19] a reaction which presumably destroys or inactivates the cellular receptors upon which the infectivity of the virus depends.

The infective particle itself is susceptible to the direct action of various agents. Exposure of influenza virus to ultraviolet irradiation produces a rapid loss of infectivity, although the capacity to be adsorbed by susceptible cells is retained for considerably longer periods.[8] The infective and eluting properties of the virus are relatively more heat labile than the hemagglutinating capacity. A number of chemical compounds has been shown to have *in vitro* virucidal activity,[5, 12, 18] but for the most part their mechanisms of action are poorly understood. In low concentrations some of these agents act as enzyme inhibitors and in high concentrations as general protoplasmic denaturants.

In an attempt to elucidate some of the factors concerned with the infectivity of influenza virus, the effect of a group of compounds of known reactivity was determined. The action of some simple oxidizing agents was studied in relation to some of the biological properties of the virus.

Materials and methods

The PR8 strain of influenza A was used throughout. Allantoic fluid suspensions of virus were prepared by inoculation of 10-day-old chick embryos by the allantoic route with a 10^{-8} dilution of passage material. After incubation at 36.5°C. for 48 hours, viable embryos were chilled at 4°C. for 4 hours and the blood-free allantoic fluids

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harvested. These fluids were pooled and stored at —76°C. in sterile, sealed ampoules; samples of pools were removed for infectivity and hemagglutinin determinations.

The infectivity of individual pools was determined by inoculation of 0.2 ml. quantities at logarithmic dilutions into each of 8 embryos. The 50 per cent embryo infective dose (EID_{50}) was calculated by the method of Reed and Muench.[15]

An embryo was considered to be infected if its 48-hour harvested allantoic fluid would agglutinate an equal volume of 0.25 per cent washed chicken erythrocytes. In all experiments, embryos receiving treated virus were compared with a similar number of controls by noting the percentage of infected individuals in each group.

Hemagglutinin and antibody titrations were performed by the method of Salk[18] and read after 90 minutes at room temperature. Chicken erythrocyte suspensions were stored at 4°C. and were not used after 6 days. All serums were inactivated at 56°C. for 30 minutes.

Dilutions of chemicals and virus were made in physiologic salt solution buffered at a pH of 7.0 with 0.25 M phosphate. All reagents used were purified or crystalline preparations and solutions were prepared just prior to use. Cytochrome C was extracted from beef heart by the method of Keilin and Hartree.[9] Allantoic fluids of uninfected embryos inoculated with solutions of compounds used in these experiments did not agglutinate chicken erythrocytes nor did they inhibit virus hemagglutination.

Experimental results

The effect of oxidizing agents on the infectivity of saline suspensions of virus. Various compounds were screened for *in vitro* action on a constant dose of virus. $KMnO_4$, $NaIO_4$, $K_3Fe(CN)_6$, and $K_2Cr_2O_7$ were used as representative inorganic compounds with relatively high oxidation potentials. O_2 and H_2O_2 were tested in order to determine the effect of increased oxygen tension on virus infectivity. *P*-benzoquinone was selected as a simple organic oxidizing agent and its action compared with hydroquinone. Because the toxicity of these compounds was a determining feature in selecting the test dose, it was not possible to make a strict comparison of their effects on the basis of equivalent concentrations from the standpoint of oxidation potentials.

The experiments were performed by diluting the virus in solutions of potential inactivators to 50 EID_{50}, the elapsed time from dilution to injection being less than 10 minutes. The effect of O_2 was determined by bubbling it into a 50 EID_{50} suspension of virus at 30 pounds pressure for 5 minutes. In each case the infectivity for chick embryos of treated virus was compared with an equal number of controls. A single pool of virus was used at a dilution of 10^{-7} in buffered saline solution of which 0.2 ml. represented 50 EID_{50}.

The results of these experiments are recorded in detail in Table 1. There was no increase in embryo mortality except with H_2O_2 which was used in one hundred times the molar concentration of the other materials. In 5 control series, totaling 101 viable embryos, the virus in saline had a mean infectivity of 95.0 per cent with a standard deviation of 4.7 per cent. Compared with this, 0.1 M H_2O_2 and 0.001 M $NaIO_4$ reduced the proportion of infected embryos to 11 and 30 per cent respectively, whereas 0.001 M solu-

tions of $KMnO_4$ or quinone completely destroyed the infectivity of the virus. All other materials were ineffective at the concentrations used.

The relative inactivating capacities of these agents were determined for varying concentrations of virus. Eight embryos were inoculated at each logarithmic dilution of virus in solutions of oxidizing agents and an equal number of control animals received virus in saline. Two other materials were used in the same manner. Cytochrome C was tested to determine if a catalytic agent for oxidation could influence the susceptibility of the tissue to infection. The effect of ammonium iodoxybenzoate was determined for

TABLE 1

PER CENT INFECTIVITY OF 50 EID_{50} OF PR8 VIRUS IN SALINE SOLUTIONS OF VARIOUS COMPOUNDS AT A pH OF 7.0

		No. of embryos each group					
Diluent	*Conc. (molar)*	*Inoculated*	*Dead 24 hrs.*	*Dead 48 hrs.*	*Infected survived*	*Mortality (%)*	*Infectivity (%)*
Saline		113	5/113	12/113	96/101	10.7	95.0
H_2O_2	0.1	50	20/50	32/50	2/18	64.0	11.1
$NaIO_4$	0.001	50	4/50	5/50	13/43	10.0	30.2
Quinone	0.001	25	2/25	3/25	0/22	12.0	0
Hydroquinone	0.001	25	1/25	1/25	22/23	4.0	95.7
$KMnO_4$	0.001	25	1/25	1/25	0/24	4.0	0
$K_3Fe(CN)_6$	0.001	25	0/25	2/25	22/22	8.0	100.0
$K_2Cr_2O_7$	0.001	25	0/25	2/25	22/23	8.0	95.7
O_2	30 lbs. 5 mins.	25	0/25	1/25	24/24	4.0	100.0

comparison with H_2O_2; both of these compounds liberate oxygen in solution. The 50 per cent infective endpoints of virus diluted in these compounds and in saline are compared in Table 2.

In the concentrations used, O_2, cytochrome C and ammonium iodoxybenzoate had no appreciable effect on virus infectivity. H_2O_2 produced a moderate reduction in infectivity at near-lethal doses; its action is attributable either to release of active nascent oxygen or to its dehydrogenating capacity. A common characteristic of the compounds that produced a significant reduction of virus infectivity is their dehydrogenating capacity. Apparently, another important factor in the activity of these compounds is the pH of the reacting medium; this probably accounts for the maintenance of complete virus infectivity in neutral solutions of $K_3Fe(CN)_6$ and $K_2Cr_2O_7$. There is some correlation between the degree of inactivation produced by $KMnO_4$, $NaIO_4$, and H_2O_2 and the oxidation-reduction potentials[13] of these compounds. However, the action of quinone must be explained on some other

basis since it is the most effective inactivator but a relatively poor oxidizing agent.

The protective action of biological material. Ostravskaya, *et al.*,[14] reported that H_2O_2 and $KMnO_4$ caused relatively little reduction in infectivity of unpurified mouse lung preparations of influenza virus. Knight and Stanley[13] demonstrated a pronounced effect of oxidizing agents, such as iodine, on highly purified allantoic fluid preparations of PR8 virus. The most plausible explanation of these contradictory findings appears to be that the degree of inactivation of virus is influenced to a great extent by the presence in the suspending medium of oxidizable biological material. Purified virus is susceptible to oxidation, whereas virus exposed in mouse lung preparations is protected by tissue debris.

TABLE 2

COMPARISON OF 50 PER CENT INFECTIVE ENDPOINTS OF PR8 VIRUS IN SALINE AND OTHER DILUENTS. (8 EMBRYOS INOCULATED AT EACH LOGARITHMIC DILUTION)

Test material as diluent	*Conc.*	*Saline control 50% log titer*	*Experimental 50% log titer*	*Log difference infectivity*	*50% EID protection*
H_2O_2	0.2 M	5.82	2.0	3.82	6,607
$NaIO_4$	0.001 M	7.09	3.81	3.28	1,906
Quinone	0.001 M	7.09	2.40	4.69	48,980
$KMnO_4$	0.001 M	7.09	2.49	4.60	38,810
Ammonium iodoxybenzoate	0.01 M	6.00	6.00	0	0
Cytochrome C	1.0 mg. per ml.	4.43	4.45	0	0
Cytochrome C	1.0 μg. per ml.	6.99	5.64	1.35	22

To determine the effect of the presence of nonspecific oxidizable material on virus inactivation, 40 per cent solutions of normal rabbit serum and normal allantoic fluid and a 5 per cent solution of glucose in saline solution were used as virus diluents. To these solutions the oxidizing agents were added at the same concentration and allowed the same time of exposure as in the previous experiments. The infectivity of 50 EID_{50} of virus in saline solution treated in the same manner was determined concurrently.

$KMnO_4$ and $NaIO_4$ were incapable of producing any significant diminution in the infectivity of virus diluted in normal rabbit serum or normal allantoic fluid. The inactivating effect of $NaIO_4$ was also blocked by glucose. In marked contrast to these protective effects was the action of quinone which completely inactivated the virus regardless of the diluent (Table 3).

In vivo effects. These results do not exclude the possibility that oxidizing agents act on the host cell rendering it refractory to virus infection. Hirst[7] has shown that erythrocytes treated with $NaIO_4$ will not adsorb influenza virus and that this receptor-destroying action could be blocked by glucose. To determine if the host cell receptor was the site of action of oxidizing agents, saline solutions of these materials were injected by the allantoic route. Ten minutes later the embryos were challenged with 50 EID_{50} of PR8 virus by the same route.

With the possible exception of quinone, which produced a 23 to 28 per cent reduction in infectivity, pre-inoculation of oxidizing agents caused no change in the susceptibility of the embryos to infection. The action of

TABLE 3

THE PROTECTIVE EFFECT OF BIOLOGICAL FLUIDS ON THE INACTIVATION OF 50 EID_{50} OF VIRUS BY OXIDIZING AGENTS (RESULTS EXPRESSED IN PER CENT INFECTIVITY)

		Diluents (final concentration)			
Inactivator	*Final conc. (molar)*	*Saline 0.85%*	*Glucose 5%*	*Normal rabbit serum 40%*	*Normal allantoic fluid 40%*
Saline		95.0	93.0	94.7	90.0
H_2O_2	0.1	11.0	9.0		
$NaIO_4$	0.001	14.0	78.0	81.2	80.0
$KMnO_4$	0.001	0	0	100.0	81.2
Quinone	0.001	0	0	4.3	0

quinone, however, is presumably largely on the virus particle, since when it was injected in the same concentration 30 minutes after the virus, it reduced the proportion of infected embryos to 55 per cent.

The antagonistic effect of reducing agents. The presence in virus suspensions of hydrogen donators equimolar to the concentrations of the subsequently added oxidizing agents should exert a neutralizing effect on their inactivating capacity. This, of course, would depend on the uniformity of action of the oxidizing agents and their site of action on the infective particle. In addition, the capacity of reducing agents to prevent virus inactivation would be a function of the reaction times of the various materials and the reduction potentials of the antagonists.

Solutions of cysteine, glutathione, methionine, and cystine at 0.002 M concentrations were used as diluents for the virus and their effects compared with that of a relatively strong reducing agent, *l*-ascorbic acid. To suspensions of 100 EID_{50} of PR8 virus in solutions of these compounds, equal volumes of 0.002 M solutions of oxidizing agents were added and then in-

jected into embryos after 10 minutes at room temperature. The infectivity of virus in solutions of saline, antagonists, and oxidizing agents alone served as controls for each series of experiments. The virus lost none of its infectivity in solutions of the amino acids, glutathione, or ascorbic acid when the pH was maintained at 7.0. Dehydroascorbic acid also had no effect. Knight and Stanley[12] reported that 0.05 N ascorbic acid reduced the infectivity of influenza virus only with prolonged exposure by changing the pH of the medium to the acid side.

Dilution of virus in ascorbic acid neutralized the inactivating effects of quinone, $KMnO_4$ and $NaIO_4$. H_2O_2, in one hundred times the molar concentration of the other oxidizing agents, was still effective in destroying the

TABLE 4

RESISTANCE TO INACTIVATION BY OXIDIZING AGENTS OF 50 EID_{50} OF PR8 VIRUS WHEN DILUTED IN SOLUTIONS OF "REDUCING AGENTS." (RESULTS EXPRESSED IN PER CENT OF INFECTED EMBRYOS, 20-25 PER GROUP)

		Antagonist as diluent				
		L-*Cysteine*	*Glutathione*	D,L-*Methionine*	L-*Cystine*	L-*Ascorbic acid*
Oxidizing agent	*Final conc. (molar)*	0.001	0.001	0.002	0.001	0.001
Saline		100.0	85.0	93.0	95.0	100.0
H_2O_2	0.1	100.0				20.0
$NaIO_4$	0.001	5.3	0			75.0
$KMnO_4$	0.001	0	14.0			88.2
Quinone	0.001	95.0	88.8	0	0	93.3
Hydro-quinone	0.001				100.0	

infectivity of the virus in ascorbic acid solution. Cysteine and glutathione failed to protect the virus against the inactivating effect of equimolar concentrations of $KMnO_4$ and $NaIO_4$. However, these weak reducing agents were completely effective in blocking the action of quinone and H_2O_2. This neutralizing effect would seem to be due to the sulfhydryl groups of these compounds since methionine and cystine had no influence on the virus inactivating capacity of quinone (Table 4).

The effect of oxidizing agents on antigenicity. The antigenic potency of influenza viruses appears to be more resistant to the effects of ultraviolet irradiation of certain wave length and intensity[17] than do the infective or hemagglutinating properties.[9]

The effect of oxidizing agents on the antihemagglutinin-producing activity of the PR8 strain was determined in rabbits. The virus was diluted in

0.002 M solutions of $NaIO_4$, $KMnO_4$, and quinone to a concentration of 10^{-3}. After standing at room temperature for one hour, 1.0 ml. of each suspension was injected intravenously into male albino rabbits weighing 2,300 to 2,500 grams. Aliquots of these preparations were inoculated into chick embryos, 1.0 ml. of the equivalent saline-diluted virus concentration represented 250,000 EID_{50}. The virus in $NaIO_4$ solution showed residual infectivity but on no occasion were the $KMnO_4$ or quinone-treated inoculums infective for embryos. Each rabbit received three doses at weekly intervals and was bled 7 days after the last injection. Fifty-six-degree inactivated pre- and postinoculation paired serums were titrated for hemagglutination inhibition.

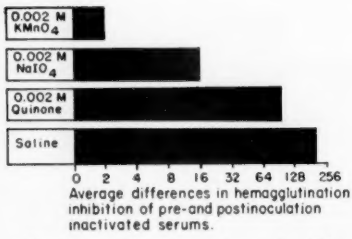
0.002 M KMnO4
0.002 M NaIO4
0.002 M Quinone
Saline
0 2 4 8 16 32 64 128 256
Average differences in hemagglutination inhibition of pre-and postinoculation inactivated serums.

FIG. 1. Antibody responses of rabbits to PR8 virus in solutions of oxidizing agents. Each rabbit received three intravenous injections of 250,000 EID_{50} at weekly intervals.

The average antihemagglutinin responses of groups of three animals are represented in Figure 1. The antigenic activity of this quantity of virus was almost completely destroyed by $KMnO_4$. $NaIO_4$, representing a lower inactivating dose, produced a moderate reduction of antigenicity. The virus preparations exposed to the action of quinone were almost as antigenic as the control virus in saline solution.

The effect of oxidizing agents on virus hemagglutinins. The hemagglutinating capacity of treated virus was determined by diluting allantoic fluid containing PR8 virus with 20 volumes of 0.01 to 0.001 M concentrations of oxidizing agents in physiologic salt solution. $NaIO_4$ solutions represented about 4 per cent of the inactivating dose of equal concentrations of $KMnO_4$ or quinone. These virus suspensions were then centrifuged at 30,000 r.p.m. for 90 minutes and each sediment was reconstituted with buffered saline solution to the original allantoic fluid volume. To serve as controls, saline suspensions of virus were treated in the same manner.

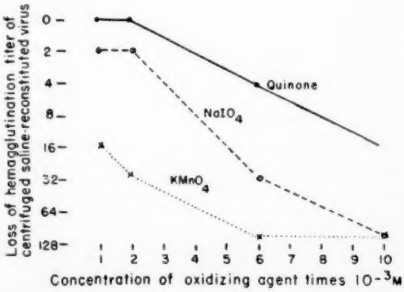

FIG. 2. Effect of oxidizing agents on PR8 hemagglutinins.

Results are represented graphically in Figure 2 as the loss of hemagglutinating activity of a constant amount of virus treated with varying concentrations of oxidizing agents. The findings again indicate a dissimilarity of

action of these agents. Quinone, which is most effective in destroying the infectivity of the virus, altered its hemagglutinating activity only slightly even in high concentrations. In minimal inactivating concentrations, $KMnO_4$, and to a lesser extent $NaIO_4$, reduced the red cell agglutinating capacity of influenza virus.

There was no detectable elution of adsorbed virus after treatment with quinone.

The effect of soluble sulfhydryl compounds on inactivated virus. Experiments were designed to determine if the effect of oxidizing agents on the infectivity of the virus was reversible.

Solutions of minimal inactivating concentrations of $NaIO_4$, $KMnO_4$, and

TABLE 5

REACTIVATION IN PER CENT INFECTIVITY OF 50 EID_{50} OF PR8 VIRUS IN SOLUTIONS OF OXIDIZING AGENTS

		"Reactivator"			
Oxidizing agent as diluent	*Final conc. (molar)*	*Saline*	L-*Cysteine* 0.01	*Glutathione* 0.01	L-*Ascorbic acid* 0.01
Saline		100.0	90.0	60.0	100.0
$KMnO_4$	0.0001	11.8	25.0	18.3	7.1
$NaIO_4$	0.0005	60.0	76.5	64.7	63.0
Quinone	0.001	0	0		0
Quinone	0.0001	29.4	67.7		
Quinone	0.00025	15.0	76.9	38.5	7.7

quinone were used as diluents for 50 EID_{50} of PR8 virus. Suspensions of the virus in these solutions were kept at room temperature for 30 minutes. Saline, cysteine, glutathione, and ascorbic acid were then added to aliquot portions and injected into groups of 25 embryos.

There was some slight indication of greater infectivity of the virus in solutions of $KMnO_4$ and $NaIO_4$ after the addition of cysteine or glutathione. Relatively high concentrations of cysteine were without effect on virus completely inactivated by 0.001 M quinone. However, cysteine, and to a lesser extent glutathione, produced a significant reactivation of virus treated with minimal inactivating doses of quinone. This capacity to restore a portion of the infective capacity of inactivated PR8 virus was not exhibited by ascorbic acid (Table 5).

Discussion

In solutions of various compounds that possess the common characteristic of oxidizing activity, the PR8 strain of influenza A virus rapidly loses in-

fectivity. These agents, H_2O_2, $NaIO_4$, $KMnO_4$, and quinone, produced varying degrees of inactivation but in general they were effective in low concentrations at neutrality. $K_3Fe(CN)_6$ and $K_2Cr_2O_7$ are active oxidizing agents in acid solution but had no effect on the infectivity of neutral buffered saline suspensions of virus. Ammonium iodoxybenzoate and O_2 were also ineffective, indication that infectivity of the virus is not affected by increased oxygen tension. The inactivation produced by the effective compounds, therefore, appears to be associated with their dehydrogenating activity in neutral solutions. It is presumed that the virus-inactivating effect of H_2O_2 is related to its hydrogen-accepting capacity. There is some correlation between relative inactivating effectiveness and oxidation potentials of $KMnO_4$, $NaIO_4$, and H_2O_2. However, quinone is a relatively poor oxidizing agent but produced the greatest reduction in virus infectivity.

The action of $KMnO_4$ and $NaIO_4$ seems to be largely a nonspecific oxidative phenomenon. Dilution of the virus with oxidizable biological material like normal rabbit serum or normal allantoic fluid blocks their inactivating effect. Glucose also protects the virus against the action of $NaIO_4$. The loss of hemagglutinating and antigenic properties of the virus in saline solutions of these agents indicates that their action has resulted in a widespread destruction of virus substance. The findings of Hirst[8] of only slight loss of hemagglutinating activity of periodate-treated Lee strain of influenza virus are probably explicable on the basis of variation in total dosage, relative differences in the presence of inert oxidizable material in undiluted specimens and to some extent strain susceptibility.[1]

In comparison with the other inactivating agents, the action of quinone is not inhibited by nonspecific oxidizable material, and when its injection precedes or follows virus challenge by a short interval, there is a slight reduction in the number of infected embryos. In concentrations sufficient to render the virus noninfective, quinone produces little alteration of antigenic or hemagglutinating properties. These results indicate that in low concentrations, quinone exhibits a selective action on the infective and eluting properties of the virus. Its greater *in vitro* effectiveness may be explained on the basis that its reactivity is partly limited to chemical groups that are concerned with virus infectivity. On the other hand, some of the inactivating capacity of $KMnO_4$ and $NaIO_4$ appears to be dissipated on virus radicals not essential for infectivity.

The neutralizing action of ascorbic acid on the inactivating effects of $KMnO_4$, $NaIO_4$, and quinone is presumably due to its strong reducing capacity. This protective effect is not exhibited for $KMnO_4$ and $NaIO_4$ by weak reducing agents like cysteine and glutathione. The quinone-inhibiting effect of these latter compounds seems to be due to their sulfhydryl moieties since methionine and cystine were not effective. The capacity of sulfhydryl compounds to neutralize the antibacterial action of quinones and hydroquinones has been demonstrated,[9] and presumably a similar chemical

mechanism would apply to their protective action against the antiviral effect of quinone. *In vitro* inactivation and modification of infection of chick embryos has been demonstrated for the PR8 strain with certain dyes of the quinone-imide group.[2]

Cysteine and glutathione were capable of restoring a significant portion of the infectivity of quinone-inactivated virus. Klein, Perez, *et al.*,[10, 11] have shown that BAL has a similar action on influenza virus treated with mercuric chloride. It seems likely that the primary action of quinone on this virus is a reversible one, its presence resulting in the formation of a noninfective but otherwise intact particle. The reactivation produced by cysteine and glutathione appears to be associated with the presence in the suspending medium of excess free sulfhydryl groups which are necessary for virus infectivity.

Summary and conclusions

In saline solutions of $KMnO_4$, $NaIO_4$, H_2O_2, and quinone, influenza A virus rapidly loses infectivity for chick embryos. With the exception of quinone, which is the most effective inactivator, the relative inactivating capacities of these compounds are correlated with their oxidation potentials.

The presence of nonspecific oxidizable material blocks the action of $KMnO_4$ and $NaIO_4$ but not of quinone.

Ascorbic acid neutralizes the action of these inactivators when equimolar concentrations are used. Cysteine and glutathione protect against the inactivating effect of quinone but not of $KMnO_4$ and $NaIO_4$.

In solutions of $KMnO_4$ there is a marked destruction of the hemagglutinating and antigenic activities of influenza A virus. The effect of $NaIO_4$ is less pronounced, whereas quinone produces relatively little alteration in these properties.

Cysteine, and to a lesser extent, glutathione are capable of restoring a significant portion of the chick embryo infectivity of virus inactivited with minimal effective doses of quinone. Under the same conditions, virus treated with $KMnO_4$ and $NaIO_4$ regains little of its infectivity.

It seems likely that $KMnO_4$ and $NaIO_4$ are effective inactivators of the PR8 strain of influenza virus by virtue of a nonspecific oxidation. In low concentrations, quinone appears to have a selective affinity for virus constituents that are associated with infectivity.

Evidence presented previously and in this report suggests that sulfhydryl groups are essential for influenza virus infectivity.

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PRELIMINARY OBSERVATIONS ON THE EFFECT OF AUREOMYCIN, TERRAMYCIN, TIBIONE, COMBINED TIBIONE AND STREPTOMYCIN, AND CHLOROMYCETIN ON THE MORPHOLOGY OF THE LIVER IN MAN*

RAYMOND YESNER AND PAUL KUNKEL

Within recent years needle biopsy of the liver has become an excellent research as well as diagnostic tool, and the observations made available by this technique have both theoretical and practical significance. With its aid, base-line observations of liver morphology may be made and periodic sampling thereafter may reflect the changes occurring in this organ as a result of drug therapy.

With the use of this method preliminary observations of liver morphology have been made before, during, and after treatment with aureomycin, terramycin, Chloromycetin, streptomycin, para-amino-salicylic acid, and tibione. Associated with aureomycin therapy fatty metamorphosis of the liver has been observed.

Aureomycin. Four cases treated with aureomycin were studied.

Case #1. M. A., a 37-year-old white male was admitted to the hospital in February 1950 with recurrent acute brucellosis proved by six blood cultures, a liver, urine, and bone marrow culture that were all positive for Brucella abortus. A liver biopsy on 2/10/50 showed numerous epithelioid granulomata in both portal triads and parenchymal areas. There was an occasional large fatty vacuole (Fig. 1). Aureomycin was started on 2/16/50 and continued for fourteen days, for a total of 43 grams. Liver biopsy on March 3, the day after completion of treatment, showed that epithelioid granulomata were still present. There were numerous small vacuoles of fatty metamorphosis, most of the vacuoles approximating the size of the liver cell nuclei (Fig. 2). Liver function studies on 2/16/50 revealed a bromsulphthalein retention of 11% and a cephalin flocculation of ++. On 2/21/50, the bromsulphthalein was 6%, the cephalin flocculation ++, and the thymol turbidity ++. On 3/2/50 the bromsulphthalein was 0%, the cephalin flocculation and thymol turbidity were negative. The patient's temperature fell to normal on the fifth day of therapy and remained so thereafter. During his stay in the hospital his weight remained constant at 205 pounds although some anorexia and nausea accompanied the aureomycin therapy. A third liver biopsy was done on April 15, 1950, 44 days after the cessation of aureomycin therapy, and revealed an occasional small intrasinusoidal collection of reticulum cells. The hepatic parenchymal cells contained only an occasional large fatty vacuole (Fig. 3).

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Case #2. S. R., a 52-year-old white male, was admitted to the hospital on February 1, 1950 with a febrile illness associated with a 25-pound weight loss and epithelioid granulomata of the lymph nodes and bone marrow. Liver biopsy done on 2/18/50, following a maximum weight loss, showed large granulomata present mid-zonally and sharply circumscribed by envelopes of fibrous tissue. There was no fat in the parenchymal cells (Fig. 4). The differential diagnosis included sarcoidosis and brucellosis. Because of the latter, although no positive cultures were obtained, aureomycin, 4.0 grams daily, was administered from March 8 through March 28, the patient receiving a total of 84 grams. A repeat liver biopsy on April 2 revealed a moderate degree of small cell vacuolization of the parenchymal cells, particularly in the central areas of the lobules. The vacuoles tended to be about the size of parenchymal cell nuclei or smaller (Fig. 5). No granulomata were present in the sections. Following cessation of all chemotherapy the patient's temperature returned to normal, he became asymptomatic, and was discharged on 6/16/50. He received no parenteral glucose and his nutrition was maintained well by mouth.

Case #3. R. L. T., a 25-year-old white male, entered the hospital on September 14, 1950 with a history of headache of twelve days' duration and drenching night sweats and fever of eight days' duration. He was a milk truck driver, and for one month prior to admission had drunk raw milk for a period of ten days. Sternal and iliac bone marrow cultures were positive for *Brucella suis.* A liver biopsy on 9/18/50 revealed numerous epithelioid granulomata in both portal triads and parenchymal areas (Fig. 6). The parenchymal cells were devoid of fat. Aureomycin was started on 9/23/50 and continued for fourteen days for a total of 94 grams. Liver biopsy on October 5, on the thirteenth day of therapy, showed that epithelioid granulomata were still present. There were numerous small vacuoles of fatty metamorphosis, most of the vacuoles approximating the size of the liver cell nuclei (Fig. 7). Cephalin flocculation on 9/18/50 was 3+ and on 9/29/50 was 2+. Prior to therapy there was an occasional spiking of temperature to 100°F. After aureomycin therapy was instituted, temperature remained normal. During the period of aureomycin therapy he ate well and gained 10 pounds. A third liver biopsy done on 10/11/50, five days after cessation of aureomycin therapy, revealed fatty metamorphosis still present, but not as extensive as in the preceding biopsy. Sudan III stain of a frozen section confirmed the interpretation of the vacuolization as lipoid droplets (Fig. 8).

Case #4. D. L. G., a 29-year-old white male, entered the hospital on June 12, 1950 with a history of fever, malaise, and jaundice of four days' duration. A diagnostic liver biopsy on 6/19/50 revealed acute cholangitis. There was no fatty vacuolization of the parenchymal cells. The patient continued to run a septic fever after admission and on 6/21/50, aureomycin, 4.0 grams daily, was administered empirically for fourteen days for a total of 56 grams. His temperature failed to respond to this therapy, but gradually fell to normal after cessation of the antibiotic. A second liver biopsy was performed on 7/21/50 and showed subacute cholangitis, early biliary cirrhosis, and bile stasis. There was no fatty metamorphosis of the parenchymal cells. Because of a recrudescence of his jaundice under observation, he was explored surgically on July 27, 1950 and chronic pancreatitis was found with enlarged lymph nodes in the region of the common bile duct. During his hospitalization his weight fell from 121 pounds to 109 pounds, and then rose to 113 pounds. His appetite was poor until the cessation of aureomycin therapy, but his nutrition was maintained by a high protein diet.

Comment. a. Four patients were treated with aureomycin. In three a fine fatty vacuolization was found immediately after 13 to 20 days of therapy. In Case #4 the post-treatment biopsy failed to reveal fatty metamorphosis but the biopsy was not done until sixteen days after treatment ceased. It is

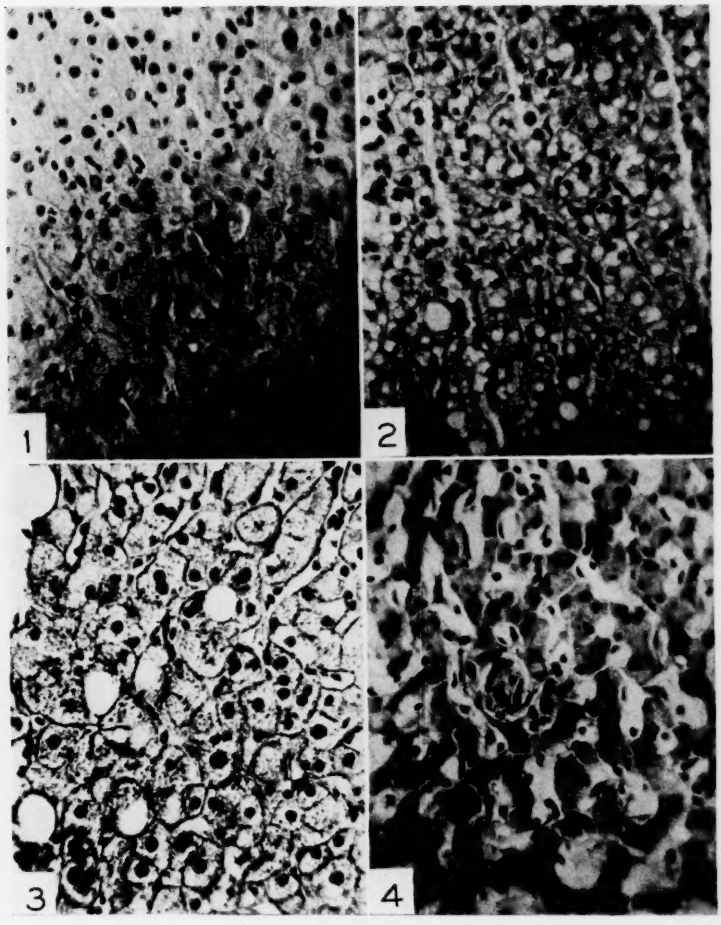

FIG. 1. M.A.: First biopsy. Small epithelioid granuloma is present; hepatic cells are devoid of fat.

FIG. 2. M.A.: Second biopsy. Marked small vacuole fatty metamorphosis is present.

FIG. 3. M.A.: Third biopsy. Occasional large fat vacuole is present.

FIG. 4. S.R.: First biopsy. Small intrasinusoidal collection of reticulo-endothelial cells is present; hepatic cells are devoid of fat.

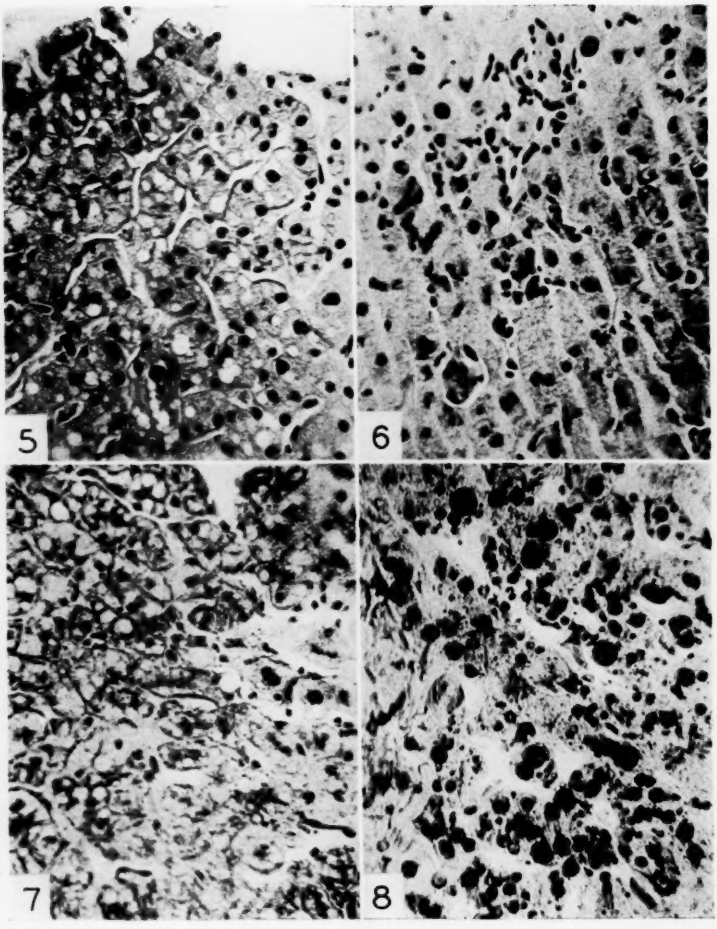

Fig. 5. S.R.: Second biopsy. Moderate small vacuole fatty metamorphosis is present.
Fig. 6. R.L.T.: First biopsy showing hepatic granulomata and no fat in the parenchymal cells.
Fig. 7. R.L.T.: Second biopsy. Diffuse fatty metamorphosis.
Fig. 8. R.L.T.: Third biopsy. Less marked fatty metamorphosis with Sudan IV stain.

possible that had the biopsy been done immediately following treatment, the fatty change might have been demonstrable.

b. In one of the three cases (Case ♯1) with fatty metamorphosis of the liver, a 44-day post-treatment liver biopsy revealed an occasional large fatty vacuole, which was also present in the pretreatment biopsy.

c. These findings suggest that aureomycin was responsible for fatty change in the liver. It is unlikely that the disease per se produced the fatty change observed in Case ♯1, a chronic relapsing disease, or in Case ♯2, a chronic disease, or in Case ♯3, an acute disease. Pretreatment biopsies in each case showed absence of fatty change. Furthermore, the biopsy in Case ♯2 was done at a time when weight loss was at a peak. The fatty change in Cases ♯2 and ♯3 occurred only after aureomycin therapy, at a time when the patients were improving clinically and were afebrile. Furthermore, it is unlikely that a sampling error occurred in these adequate biopsies, because it is our experience that when fatty change occurs in the liver it is of remarkably uniform lobular distribution. Waldstein and Szanto[3] have also recently demonstrated that with respect to fatty metamorphosis and injury of the epithelial liver cells, multiple needle biopsy specimens are almost always uniform throughout the liver.

d. In Case ♯2 the patient developed a mild transient cheilosis at the angles of the mouth for one week immediately following the cessation of aureomycin therapy. This is similar to the riboflavin deficiency described by Harris.[1] The mechanism involved is either an interference with the absorption or utilization of riboflavin because of the induced change in the intestinal flora, or a defect in utilization caused by aureomycin's interfering with the enzyme systems. It is possible that similar mechanisms may cause a deficiency of lipotropes in association with aureomycin therapy, resulting in fatty metamorphosis of the liver. There was no clinical evidence of vitamin deficiency in Cases ♯1 and ♯3.

Terramycin. In the following case, the effects of terramycin therapy were noted.

Case ♯5. T. M. W., a 32-year-old white male with typhoid fever was given 28.5 grams of terramycin from August 26 to September 4, 1950. Liver biopsy on the sixth day of terramycin therapy, after 17 grams, revealed absence of fatty metamorphosis.

Klatskin[2] has found fatty metamorphosis, similar to that seen following aureomycin therapy, in one case two days after cessation of terramycin therapy, in which 72 grams were given over a 16-day period. Control liver biopsy four days prior to terramycin therapy revealed no fat. In a second case, marked fatty metamorphosis of the liver was seen following the administration of 31 grams of terramycin over a 9-day period. The control liver biopsy one day prior to the institution of therapy was normal. This is in contrast to Case ♯5, in which no fatty metamorphosis was found in the liver at a time when only 17 grams of terramycin over a period of six days had been administered.

Tibione. In the following three cases the effects of tibione therapy alone were evaluated. This study was undertaken during the course of an investigation of the toxicity of tibione.

Case #6. R. D., a 57-year-old white male with old pulmonary tuberculosis was admitted on July 14, 1949 for decortication of the right lung. Following his decortication, which was done without benefit of antibiotic therapy, he was placed on a 120-day course of tibione, receiving 300 mg. a day. The liver biopsy on December 30, 1949, before tibione was started, verified the clinical diagnosis of cirrhosis of the liver. The parenchymal cells contained only the very smallest amount of lipoid vacuolization as shown by the presence of a few tiny vacuoles in the periportal areas. The second liver biopsy was done on May 2, 1950. The histologic appearance was identical with that of the previous biopsy with the exception that the trace of fatty vacuolization had disappeared.

Case #7. H. E., a 64-year-old white male who died as a result of widespread carcinoma of renal origin, had a total of 35 days of treatment with tibione, 200 mg. a day. The liver biopsy on December 12, 1949, done prior to treatment, revealed normal hepatic tissue. At autopsy on February 16, 1950, the liver revealed marked invasion by metastatic renal carcinoma. The uninvolved parenchyma was devoid of fatty vacuolization or other changes.

Case #8. G. U., a 52-year-old white male, was admitted in delirium on November 17, 1949 with alcoholic cirrhosis. This patient received 90 days of tibione therapy in amounts of 200 mg. a day. The first liver biopsy on December 28, 1949, before receiving tibione, revealed Laennec's cirrhosis. No fatty vacuolization was present. The second and third liver biopsies on March 3, 1950 and on April 24, 1950, revealed no change in the cirrhosis and no fatty metamorphosis. Groups of regenerating liver cells were present in the third biopsy.

Comment. In these three cases treated with tibione alone no evidence of morphologic change was seen in the liver parenchyma.

Tibione, streptomycin, and para-amino-salicylic acid. The following three cases with active tuberculosis were given tibione with various combinations of streptomycin and para-amino-salicylic acid.

Case #9. R. E. B., a 30-year-old white male with bilateral far advanced upper lobe tuberculosis was given tibione for a total of 142 days, 200 mg. a day, and streptomycin, 1 gram a day. The first liver biopsy obtained on January 13, 1950, before tibione was started, revealed entirely normal hepatic tissue without any fatty metamorphosis. The second biopsy obtained on June 15, 1950 also revealed hepatic tissue that was entirely normal without any fatty metamorphosis.

Case #10. G. J. M., a 36-year-old white male with tuberculous synovitis was given tibione for a total of 131 days, 200 mg. a day, and streptomycin, 1 gram every three days. The first liver biopsy done prior to starting tibione therapy on February 9, 1950, revealed a normal lobular architecture. The parenchymal cells were normal and devoid of fat. The second liver biopsy on June 23, 1950, performed nine days after the cessation of tibione therapy, revealed the histologic characteristics of infectious hepatitis. On April 25, 1950 this patient complained of anorexia and vomited occasionally. Since there was no icterus, enlargement of the liver, nor tenderness to percussion in the right upper quadrant, the patient was continued on tibione. Symptoms disappeared on May 23, 1950. On June 5, 1950 the bromsulphthalein retention was 14½%. On June 16 the bromsulphthalein retention was 22% and on June 28 the bromsulphthalein retention was 0%. Tibione was discontinued on June 17. This patient

had a synovectomy performed on March 22, at which time he received one pint of bank blood. This case is being considered as a non-icteric viral hepatitis, possibly homologous serum hepatitis occurring during a course of tibione but unrelated to the latter. This conclusion is based on the symptomatic improvement that occurred in spite of the continued administration of tibione and the characteristic histopathology of the liver in this disease.

Case #11. T. E. L., a 60-year-old white male with far advanced bilateral upper lobe tuberculosis with cavitation was given tibione for a total of 90 days, 200 mg. a day, and streptomycin, 1 gram a day. The first liver biopsy done prior to starting tibione therapy on April 21, 1950 revealed a normal hepatic architecture. The parenchymal cells were entirely devoid of fat. The second biopsy done on July 25, 1950 revealed that the liver tissue was essentially unchanged. There was no fatty vacuolization.

Case #12. J. P., a 34-year-old white male with bilateral far advanced apical tuberculosis, received a total of 120 days of tibione therapy, 200 mg. a day, streptomycin, 1 gram a day, and para-amino-salicylic acid, 13 grams a day. The first liver biopsy done on 3/28/50 showed a tubercle located within a portal triad. A minimal trace of fatty vacuolization was present in the parenchymal cells. The second biopsy was done on April 3, 1950. There were no granulomata. The parenchymal cells were entirely devoid of fat. The third biopsy done on 7/25/50 showed a normal hepatic architecture.

Comment. One case treated with combined tibione and streptomycin (Case #10) developed hepatitis, which was probably homologous serum jaundice since it subsided during continued treatment. In the two other cases treated with combined tibione and streptomycin, no evidence of morphologic change was seen in the liver parenchyma. In the one case treated with tibione, streptomycin, and para-amino-salicylic acid, there was similarly no morphologic change in the liver.

Chloromycetin. The following case was treated with Chloromycetin.

Case #13. A. S., a 28-year-old white male, was admitted to the hospital on July 11, 1950 because of an acute osteomyelitis of the right tibia. Incision and drainage of the osteomyelitic abscess were carried out on 7/12/50 and culture of the pus obtained revealed a pure growth of *Aerobacter aerogenes.* Chloromycetin (chloramphenicol), 2.0 grams daily, was started on 7/14/50. After the patient had received a total of 19.5 grams of Chloromycetin, a liver biopsy was performed on 7/24/50 and revealed normal hepatic tissue. No fatty metamorphosis was present.

Summary

1. Three of four cases treated with aureomycin showed fine fatty metamorphosis of the liver at the end of thirteen to twenty days of treatment. Forty-four-day post-therapy biopsy in one of these revealed loss of fat.

2. One case treated with terramycin showed no fatty metamorphosis of the liver at the end of six days of therapy. Klatskin[9] has observed fatty metamorphosis in two patients following a larger quantity administered over a longer period.

3. Seven cases treated with tibione and various combinations of tibione, streptomycin, and para-amino-salicylic acid showed no such change, nor

did one case treated with Chloromycetin. Despite the severe debilitating febrile courses associated with anorexia and marked weight loss in the patients with tuberculosis, no fatty metamorphosis of the liver similar to that following aureomycin therapy was seen.

4. The findings suggest that aureomycin may induce a reversible fatty metamorphosis of the liver.

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THE PLACE OF BIOCHEMISTRY IN THE UNIVERSITY*

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During the past quarter century, biochemistry has provided valuable knowledge of the nature of the chemical constituents of living matter and of their manifold transformations in the tissues of animals, plants, and microorganisms. The application of this knowledge has led to far-reaching advances in medicine and agriculture, and these contributions to human welfare and to economic progress have underlined the important place that biochemistry has assumed among the scientific disciplines.

As its name implies, biochemistry links the physical and the biological sciences. The problems which it seeks to solve are those of the biological sciences; the experimental methods and conceptual generalizations it applies to the solution of these problems are drawn from chemistry and physics, as well as from biology. Two separate streams of knowledge thus nourish the growth of biochemistry; for the biochemist, new advances in the understanding of the properties of inanimate matter have an importance equal to the discovery of new functions—and dysfunctions—in biological systems. The rapid rise of biochemistry in recent decades is the direct consequence of the fruitful blending of these two broad lines of experimental endeavor.

The major trend in current biochemical thought is the emphasis on the dynamics of chemical reactions in living systems. For this reason, especial importance is assigned to the study of enzymes, the catalytic proteins which promote and direct the myriad chemical processes essential for the maintenance of life. Attention is given to the mode of enzyme action, the manner in which enzymes co-operate in living cells, and the factors that may influence their catalytic activity. These studies have already clarified, to some degree, the biochemical role of the vitamins, many of which are now known to be closely related to participants in key enzyme-catalyzed processes. One of the important aims of present-day research in biochemistry is to link endocrinology more directly with enzyme chemistry, since it appears likely that many of the hormones may exert their dramatic action as regulators of metabolism by their influence on the rate of important chemical reactions mediated by enzymes.

Among the most significant successes of modern biochemistry has been the elucidation of the nature of the enzyme-catalyzed reactions involved in the metabolic utilization of carbohydrates and fats. The clearer understanding of these processes, which provide the principal sources of the energy essential for the maintenance of life, has profoundly influenced biochemical thought. Much effort is now devoted to the study of the chemical mechan-

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isms whereby the energy arising from the breakdown of foodstuffs is used for chemical and physical work in living systems.

In the study of biochemical dynamics, the application of isotopic compounds has provided an experimental technique by means of which there has been gathered a rich harvest of data on the sequence of chemical reactions in the metabolic transformations undergone by proteins, fats, carbohydrates, and nucleic acids. Through his use of stable and radioactive isotopes, the biochemist has been brought into intimate contact with nuclear chemistry and with the physical instruments developed for work in this field.

The current emphasis on the dynamic aspects of biochemistry, and their relation to problems of biology, medicine, and agriculture, has perhaps tended to obscure the large gaps that still exist in our knowledge of the intimate structure of the most important chemical constituents of living matter, the proteins and the nucleic acids. It must be stressed, for example, that at the present writing no preparation of a nucleic acid has been described that can be considered to be composed of a single chemical species. Though much can be learned about the dynamics of the metabolism of proteins and nucleic acids with the structural data on hand, a more complete and useful understanding of these processes can come only when there has emerged a more detailed picture of the complex chemical constitution of these important groups of substances.

Perhaps the most significant attribute of modern biochemistry is the increasing documentation of the concept that, on the biochemical level, there is a striking unity among the manifold forms of living matter. A chemical process studied in a yeast culture may therefore illumine a comparable series of reactions in mammalian muscle; or the study of the respiratory pigments of invertebrates may provide basic data for the elucidation of a general mechanism of biological oxidation. Although there is much diversity in the chemical activities of different biological forms, it is becoming ever clearer that the fundamental biochemical reactions underlying cellular function exhibit a striking uniformity from the lowest to highest forms of life. The biochemistry of the present is, therefore, "general biochemistry." As a result, the modern biochemist must be catholic in his biological interests; he may wish to use as his biological material a microorganism, or a plant, or slices of rat kidney, if he is led to believe that one of these will best serve his purpose in the study of a biochemical process.

It is an obvious abstraction to refer to a "biochemist" who is, at once, an omniverous biologist, with practical experience ranging from botany to clinical medicine; a skilled chemist, with a command of organic chemical techniques and a working knowledge of physical chemistry; and an instrumentalist, with an understanding of the details of a variety of physical apparatus, from a mass spectrometer to an electrophoresis assembly. The ability to meet these varied needs of modern biochemistry must, of neces-

sity, be divided among a large number of individuals, whose early training, in addition to a sound grounding in general biochemistry, has involved particular emphasis on some biological, or chemical, or physical aspect of this broad field. Their collective knowledge, if applied to the solution of particular biochemical problems, then may co-operate to attack these problems from several points of view, and with several experimental techniques, but with a single purpose.

It is against the background of this scope of modern biochemistry that its institutional needs should be evaluated. Clearly, the development of programs of research and instruction in general biochemistry is the responsibility of the large universities. Within the framework of departmentalization that characterizes American universities, however, biochemistry is most frequently found as one of the "preclinical" disciplines in schools of medicine. The primary teaching function of such departments is in connection with professional education leading to the M.D. degree. In a similar way, biochemical departments are attached to schools of agriculture to give instruction to students in this area of applied biology. Many of the biochemistry departments in medical schools had to be established because of a geographical separation between the medical school and the science departments of its parent university. However, even where such physical separation is negligible, biochemistry still is largely found in the medical school, or in the school of agriculture, rather than among the science departments of the university. In view of the large demand for adequately trained biochemists, many of these biochemistry departments in the professional schools have assumed a dual function in offering instruction to graduate and undergraduate students in the Faculty of Arts and Sciences, as well as to students of medicine or agriculture.

It may be said, therefore, that although biochemistry has attained the status of an independent discipline, the number of American biochemistry departments which have as their main function training and research in general biochemistry is very small indeed. In a very few instances an attempt has been made to meet this need by the establishment of a division of biochemistry which is administratively part of a university chemistry department. Some universities offer biochemical instruction in their biology departments. The confusion becomes complete when one finds, in a single university located in the same city, elementary biochemistry courses offered by three different departments.

This indefinite status of general biochemistry in American universities is probably a consequence of the fact that it is a relative newcomer to the hierarchy of scientific disciplines. It would seem, however, that a re-evaluation of the place of biochemistry in the university cannot be long delayed. In a consideration of this problem, one may well take cognizance of the notable success achieved at the University of Cambridge by the late

Sir Frederick G. Hopkins. In a memorable address[1] delivered in 1926, in which he urged the establishment of independent departments of biochemistry separate from institutes of animal physiology, Hopkins went on to say:

> The greatest need of biochemistry at the moment in my opinion is equipment which shall make possible the study under one roof (of course, from its own special standpoint alone) of all living material. No full understanding of the dynamics of life as a whole, no broad and adequate views of metabolism, can be obtained save by studying with equal concentration the green plant and micro-organisms as well as the animal. . . .
>
> Such Institutes of General Biochemistry would have to be equipped for teaching as well as for research, for we have to prepare a future generation to bear burdens greater than our own. Modern physical chemistry, and modern organic chemistry . . . are to provide entirely new concepts for biochemistry. There must be experts to understand and apply them; a task which will be impossible for those who must continue to concern themselves with the rapid growth on other sides of physiology and biology, as it will be for the equally preoccupied pure chemist.

This is the philosophy that has animated the Biochemical Laboratory at Cambridge, and no one familiar with the recent history of biochemistry will fail to acknowledge the profound influence Hopkins and his group exercised on the development of this field not only in England, but the world over. In such an evaluation, those personal qualities which won him not only the admiration and respect, but also the affection of his students must find their place. It may well be that the spirit of the Hopkins laboratory was a unique phenomenon and did not arise solely from its organizational form. The fact remains, however, that until recently its example was not emulated in the development of biochemistry in those American universities whose scholarship is comparable to that of Cambridge.

However, the point of view advocated by Hopkins also found expression in the United States. In 1906, even before the establishment of the Cambridge Laboratory, Russell H. Chittenden called attention to "the growing needs of the broad group of biological sciences, which year by year are demanding more profound recognition of their chemical aspects and problems. A new biological chemistry is arising, to contribute its explanations in the study of the dynamics of living matter. Botany, zoology, bacteriology are creating new fields for chemical research, which in turn finds unexpected practical applications. . . ."[2] These words gain especial importance if one recalls that Chittenden established the Laboratory of Physiological Chemistry of the Sheffield Scientific School in 1874, and Yale thus became the first American university to give recognition to the appearance of biochemistry as a scientific discipline.

In a consideration of the manner in which general biochemistry might be assigned its proper place in the American university, no uniform formulae

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[1] Hopkins, F. G.: *Skand. Arch. Physiol.*, Berl., 1926, *49*, 33.

[2] Chittenden, R. H.: *History of the Sheffield Scientific School of Yale University 1846-1922.* New Haven, Yale University Press, 1928, II, p. 438.

can be offered; each educational institution has problems peculiar to it, and the course of action will vary. Some general reflections may be suggested, however, as a basis for further discussion.

Clearly, the first step must be the recognition, on the part of the university administration, of the importance of biochemistry as an independent scientific discipline, quite apart from its practical applications in medicine or agriculture. Such recognition will, it is hoped, lead to the establishment of departments of general biochemistry or to the enlargement of existing university departments (e.g., now in the medical school) to permit an effective program of teaching and research in general biochemistry. The administrative difficulties that may arise when two departments operate in the same area of knowledge are obvious. For this reason it may be preferred to assign to a single university department of biochemistry the task of giving instruction to first-year medical students, as well as the larger responsibility for teaching and research in general biochemistry.

Whatever the organizational form of a biochemistry department may be, its vitality depends on a close articulation, not only on the administrative level, but perhaps more importantly, on the intellectual level, with men in all the science departments—chemistry, physics, botany, zoology, microbiology, pathology, physiology, etc.—whose interests meet on the common ground of biochemistry. The scope of biochemistry is too large today for all biochemical research at a major university to be confined to a single department. It is natural to find an organic chemist in a department of chemistry engaged in work on nucleic acids, or a zoologist who is exploring the chemical processes associated with morphogenesis, or a pathologist who is studying the biochemical aspects of renal disease. It appears important, however, that the university department of biochemistry serve as a center to bring together the varied biochemical interests of men in other departments. In some instances, it may be desirable to give such men joint appointments in biochemistry. This hybridization can only be a fruitful one, for it will strengthen the teaching and research activities of both departments involved.

In order for a department of general biochemistry to serve as a focus for university interest in this subject, it must have strength within itself, quite apart from what might be added through joint appointments. Of especial importance is that the research interests of the full-time members of the biochemical staff be of sufficient variety so that a significant area of biochemistry is within the scope of their collective experience. Among the lines of research that might be followed, these seem worthy of mention: (1) the metabolism of animals, plants, and microorganisms, as studied by various techniques, including isotopic methods; (2) the chemistry of proteins and nucleic acids; (3) enzyme chemistry; (4) microbiological chemistry; (5) chemistry and mode of action of the hormones; (6) metabolic function

of vitamins. It is obvious that a single department may not embrace all of these facets of biochemical research. However, the broader the distribution of research interest, the greater will be the contribution of the department to the intellectual life of the university. In the last analysis, of course, the development of such a broad program depends largely on the caliber of the men and women on the staff of the department. Given adequate facilities and time for research, and the stimulus from discussion with their colleagues and students, good investigators may be expected to push their projects forward with energy and enthusiasm.

Though the material contributions of biochemical research have already been great and promise to be greater, the university biochemist is likely to find his greatest satisfactions in fundamental studies, which have as their ultimate goal the understanding of "the physical basis of life." Indeed, it would seem that nearly all of the significant practical applications of biochemistry, as of other branches of science, had their origin in the so-called "pure" research which flowers best in the intellectual freedom of a great university.

Breadth of research interest will inevitably find expression in the teaching program of the department, since the two are closely dependent on one another. In general, a university department of biochemistry will have to offer a basic one-year course for first-year graduate students and qualified undergraduates majoring in biochemistry. In addition, the department may be expected to offer special courses in elementary biochemistry designed for medical students or students of agriculture, whose scientific background is inadequate for admission to the graduate biochemistry course. At the advanced level, there will be lecture, seminar, tutorial, and laboratory courses, whose nature will be determined, in large part, by the research interests of the members of the departmental staff.

It has been said that the aim of a university should be to provide a center for the fusion of scholarly research, imaginative education, and technical training. An active department of biochemistry may be expected to reflect, to a high degree, this triunal role of the university. With generous and understanding support from the administrative officers of the institution and from individuals and agencies outside the university, such a department will develop, through trial and error, a distinctive program of research and instruction on a rapidly expanding frontier of knowledge important to human welfare.

COLLATERAL RESPIRATION IN THE NORMAL AND DISEASED LUNG*

GUSTAF E. LINDSKOG

Until 1930 the pulmonary lobule was generally considered to be anatomically and functionally an end structure, to which the only access of air lay in the direct bronchial route. All available anatomical data from such pioneer workers as Joseph Marshall Flint, Macklin, and William Snow Miller argued against the possibility of air passing collaterally from one pulmonary lobule to an adjacent one through indirect channels in the periphery, when the direct bronchial route for the ingress of air had become obstructed.

In 1929, Van Allen, Lindskog, and Richter[9] were led to investigate this problem because of certain observable discrepancies in the results of bronchial obstruction. Specifically, it was noted that obstruction of a bronchus beyond the second order, whether produced by natural disease or artificially, did not *invariably* result in atelectasis. The results of their experiments led to the then novel conclusion that the pulmonary lobules or segmental areas in man, dog, cat, and certain other lower animals were not functionally end organs from the standpoint of ventilation (external respiration). Furthermore, they demonstrated that after the direct bronchial route was obstructed artificially, an indirect and peripheral route for exchange of air and maintenance of inflation from adjacent directly ventilated segments did exist. To this phenomenon was given the name *collateral respiration.*

The conclusions of these authors were based on both *in vitro* experiments with excised lung specimens, and *in vivo* observations of the normal lung by means of appropriate cannulation. The demonstrations employing excised human autopsy and animal lung specimens were simple, direct, and conclusive. An excised lobe was prepared by careful dissection at the hilus with simultaneous cannulation of a proximal branch bronchus and the main lobar stem. The lobe to be studied was then placed in a negative pressure chamber or respirator and maintained at a controlled inflation of around —10 centimeters H_2O. The two cannulae were led outside the respirator through airtight vents. A gentle stream of air regulated at very low pressures was then directed into one cannula (either in the side bronchus or the main stem), and the other cannula end was set under a water seal. Beginning at a low pressure level, usually one to two centimeters of water, a stream of gas bubbles was seen to issue from the second cannula through

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the water seal. This phenomenon was subsequently more dramatically demonstrated by insufflating[7] cigarette smoke gently into one segmental cannula, and seeing it issue promptly from the other. In no experiment using these very light pressures was there evidence of artefacts such as interstitial emphysema, subpleural bleb formation, or rupture of the pulmonary parenchyma.

To study the intact lung in the living animal, the technique was somewhat more complicated. A special method of cannulation was developed. After general anesthesia of the animal had been induced, a tracheotomy was performed, and a large glass tracheal cannula introduced. Thereafter a specially designed and prepared dilatable bronchial cannula[8] was introduced into a lower lobe (right or left) after the level for its introduction had been determined by direct bronchoscopic measurement. The cannula was dilated by a screw mechanism to fix its distal extremity tightly in position, and the proximal cannula opening was connected with a simple waterseal valve, either inspiratory or expiratory in type. When this cannula was fixed at such a level as to obstruct *an entire lobe* or *group of lobes,* no passage of air was observed through the cannula and water valve after the first two or three expirations had expelled the tidal and possibly some reserve air. In contrast, when the cannula was set in the intralobar position for the same animal so that at least *one* proximal segmental or branch bronchus remained unobstructed, each expiration resulted in the passage of several bubbles of air from the occluded lung through the cannula and out the water valve for as long as one cared to pursue the experiment. If the water valve was reversed to permit only inspiration and not expiration, the same passage of air occurred as long as the cannula was intralobar in position, but when an entire lobe was obstructed, the process of air intake was rapidly terminated with the production of obstructive emphysema and a sharp rise in lobar intrabronchial pressures and with eventual embarrassment to the animal due to mediastinal shift.

Having established the existence of collateral respiration, more detailed experiments on the mechanism, on the aerodynamics, on the chemical nature of the transpired gases, and on factors which promoted or depressed the activity of the mechanism were carried out. Some of these are still the subject of investigation.

It was soon apparent that collateral respiration (or ventilation) was not dependent on the integrity of the circulation, because it occurred in the excised lung and also in the living lung after the pulmonary artery had been ligated. Particulate matter, such as a suspension of carbon black, could not be forced from one segment to its neighbor by any physiological degree of pressure, whereas aqueous solutions of dye were easily passed. This suggested that the route for collateral exchange lay in anatomical defects of the alveolar walls, such as might be found in the so-called pores of Kohn.

By attaching small water manometers, one to the bronchial cannula

system and one to a free-lying endobronchial cannula placed proximal to the dilating cannula, pressure relationships were recorded as they resulted from or were responsible for the collateral respiratory phenomenon.[7] With the dilated bronchial cannula in the intralobar position (collateral respiration in operation) the distal pressure during *inspiration* was always slightly negative (as compared with the proximal pressure in the non-obstructed bronchus) and during expiration slightly positive. This difference was rarely more than 1.0 centimeter with quiet breathing, and it neither increased nor decreased with the passage of time as long as uniform depth of respiration was maintained. This gradient of 1.0 to 1.5 centimeters (water) thus represented the pressure necessary to actuate the peripheral collateral pathway *in vivo*. It was quite similar in magnitude to that originally observed in the *in vitro* experiments. Actually the mechanism must be even more sensitive than indicated by these figures since the cannula, rubber tubing, and water valve all introduced an element of resistance and inertia.

The attachment of a miniature and delicately balanced Krogh spirometer to the expiratory type water valve made it possible to collect samples of the collaterally transpired gases in order to determine their minute volumes and their chemical composition, and to compare the latter with simultaneous venous and arterial blood gas samples.

Turning first to the matter of minute volumes and the relative efficiency of the collateral pathways as compared with the direct bronchial route, a number of observations on four different dogs were made.[8] The total (tracheal) respiration of the animal was measured by using a closed recording spirometer system attached to the tracheal cannula. Simultaneously collecting and measuring the collaterally respired gas from an obstructed segment, it was found that the ratio was in the order of a hundred to one. Since the relative volumes of freely ventilated to collaterally ventilated lobar segments were estimated to be roughly ten to one, this gave a relative efficiency of 10 per cent for the indirect (collateral) route. Recent work on this point using rabbits[9] has given much higher values, ranging around 35 to 40 per cent.

Very interesting data developed when the oxygen and carbon dioxide tensions of the collaterally respired gas were determined. Carbon dioxide tensions varied from 34 to 48 mm., and oxygen from 75 to 98 mm. in four subjects. There was reasonable correlation with the CO_2 and oxygen tension of the blood samples taken simultaneously from the femoral artery.[8] In contrast with these findings, when the bronchial cannula was situated so as to obstruct an entire lobe (thus without possibility of collateral respiration) the gas in the obstructed lobe rapidly lost oxygen and gained carbon dioxide until levels nearly in equilibrium with mixed venous (right heart) blood were obtained—44 to 56 mm. carbon dioxide and 40 to 48 mm. oxygen. The dissociation curve of oxyhemoglobin is such that blood flowing

back to the left heart from a totally obstructed lobe would be only 70 to 75 per cent saturated; whereas blood returning from an area of lobular obstruction, with collateral respiration in action, would be 90 to 95 per cent saturated with oxygen. These data emphasize the considerable conservative function exercised by collateral respiration in preventing significant arterial oxygen desaturation in clinical disease associated with multiple bronchiolar obstructions (as with mucoid secretions). An explanation is afforded why patients with diffuse exudative lesions in the pulmonary parenchyma may sometimes present little or no arterial oxygen desaturation and therefore no cyanosis.

After the close relationship of collaterally respired gas to true alveolar air had been established, the factors which will decrease or abolish the collateral passage of air to and from an obstructed lobule or segment were studied. The peripheral nature of the mechanism suggested that pulmonary deflation alone would act in this direction, and such proved to be the case. Deflation of an excised and cannulated lung specimen increased the minimum pressures necessary to initiate collateral respiration from 1.0 cm. H_2O (fully inflated) to 10 cm. or more (partly deflated). Artificial pneumothorax induced on the side of the cannula placement in the living animal reduced or even abolished collateral respiration, as did the initiation of a phrenic paralysis.

Recently the action of histamine as a blocking agent for this mechanism has been demonstrated.[3] The intravenous injection of 0.1 to 2.0 milligrams of histamine diphosphate in dogs weighing 12 to 15 kilograms abruptly (within a second or two) terminates the most active collateral breathing. A recovery period of one to thirty minutes then gradually ensues. If the animal is protected by an antihistaminic agent (Benadryl or Pyribenzamine) prior to the injection of histamine, the depressive effect is prevented, and there may even be a slight augmentation of collateral air passage. Several clinical correlations of this observation suggest themselves. It is possible that postoperative pulmonary hypoventilation, with its attendant hypersecretion, and subsequent atelectasis, may be related to a release of histamine-like substances from areas of surgical trauma. Furthermore, the occasional alarming or even fatal bronchospastic respiratory accidents attributable to *curare* "anesthesia" may be due to this mechanism in association with the marked muscular depression; for it is now established that curare releases histamine locally at its site of action.[4]

Having demonstrated the efficacy of collateral respiration in *preventing* lobular atelectasis, an attempt was made to determine its role in the reinflation of atelectasis after removal of the causative obstruction. In previous experiments[6] it had been determined that threshold pressures of 12 to 14 centimeters of water were required to initiate re-expansion in acutely atelectatic lung from several species (cat, dog, or human fetus); the process of re-expansion was complete at 16 to 18 cm. H_2O. In order to compare

the direct with the indirect or collateral route of reinflation, animals were prepared in which both lower lobes had been rendered completely airless by bronchial irrigation with oxygen followed by ligation of the lobar bronchi. After an hour the two acutely atelectatic lower lobes were excised. The right lower lobe was then cannulated directly through the lobar bronchus, but the left only through the apical side bronchus, the main basilar bronchus being completely ligated. The two lobes thus prepared were then placed in the same respirator chamber and controlled reinflation begun. Aeration was observed to begin in one lobule of the right lower lobe, and in the cannulated apical segment of the left lower lobe at —12 cm. water. Re-expansion was complete in the right lobe at —16 and in the left at —18 cm. water.

This experiment explains why endotracheal aspiration with a catheter or bronchoscopic suction instrument in cases of postoperative atelectasis can be so effective a therapeutic measure. It seems quite unlikely that these procedures clear out *all* secretions in the smaller peripheral bronchi. They do, however, frequently open up the larger proximal and segmental branches. This permits collateral ventilation to operate, delivering air to still obstructed segments. By building up tidal and reserve air beyond the obstruction the cough mechanism may again become effective.

Within the past two years the existence of collateral respiration in the living human has been verified by appropriate cannulation.[4] The evidence seems now complete in both human and lower animals for a peripheral pulmonary mechanism which provides an alternate and indirect route for air transport when the *direct* bronchial channels of a lobular or segmental group are obstructed. It is probable, although not proved, that the collateral route is transalveolar through natural stomata in contiguous alveolar walls. Thoracic surgeons appreciate that in carrying out segmental resections in human cases this mechanism may at times produce a technical tribulation by masking intersegmental dissection planes during controlled inflation by the anesthetist.

In summary, the mechanism of collateral respiration is a conservative one, quite comparable to the well-accepted phenomenon of collateral circulation. Collateral respiration lessens the incidence of obstructive atelectasis. It equalizes pressure changes in the lung and tends to equalize alveolar gas tensions of oxygen and carbon dioxide, thus promoting a more uniform oxygenation of the venous blood. Collateral respiration furthermore provides air for effective cough in areas of established lobular obstruction. It is a delicate mechanism actuated by low pressure differentials (in the magnitude of one centimeter water, more or less) and is therefore easily disturbed by hypoventilation, pulmonary deflation, edema, inflammation, and bronchospastic agents, such as histamine. Its existence and ultimate conservative value are not prejudiced or disproved by the frequently observed clinical occurrence of atelectasis any more than the concept of

collateral circulation is vitiated by the occurrence of gangrene in an extremity after vascular injury.

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EFFECTS OF PROLONGED ALCOHOL INGESTION ON THE LIVER OF THE RAT UNDER CONDITIONS OF CONTROLLED ADEQUATE DIETARY INTAKE*

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For over a century clinicians and pathologists have recognized a relationship between over-indulgence in alcohol and the occurrence of cirrhosis, but attempts to demonstrate a direct hepatotoxic effect of ethyl alcohol or to produce alcoholic cirrhosis experimentally in animals have generally been unsuccessful. Moreover, as the result of recent studies on the hepatic effects of dietary deficiency, alcohol has been relegated to a secondary rôle in the pathogenesis of cirrhosis.

Connor[9] observed that long-standing fatty infiltration of the liver gave rise to cirrhosis, and suggested, largely by inference from studies in diabetes, that alcohol produced such infiltration by interfering with carbohydrate and fat metabolism in the parenchymal cells. However, he also recognized the importance of concomitant malnutrition as a factor in this process. Recently, emphasis has shifted from the possible cytoplasmic and metabolic effects of chronic alcoholism to the consequences of the dietary deficiencies often associated with it, and, on the basis of clinical and experimental evidence, fatty cirrhosis has come to be regarded as a manifestation of dietary deficiency related to the erratic eating habits of alcoholics.[12]

There is convincing experimental evidence that fatty infiltration and cirrhosis of the liver can be produced in animals by diets deficient in choline and its methionine precursor in protein, and that such effects can be prevented or cured by appropriate dietary supplements.[6, 11, 13, 18, 19, 26] In man there appears to be a similar relationship between dietary deficiency and the development of fatty cirrhosis, although the evidence is by no means as conclusive. The frequent occurrence of cirrhosis in non-alcoholic malnourished populations[9, 10, 28] is, perhaps, the best evidence that can be adduced, but even here certain features, such as the prominence of pancreatic lesions in some groups[9] and the frequent occurrence of hemochromatosis in others,[10] suggest that factors other than a deficiency of lipotropic substances may be operative.

In the case of cirrhosis associated with chronic alcoholism, the frequency with which signs of malnutrition can be demonstrated, and the therapeutic effectiveness of dietary supplements of protein and lipotropic substances,[5, 7, 21, 22] even in the face of continued alcohol consumption,[22, 27] constitute the evidence on which is based the current opinion that the nutritional factor is primary in its pathogenesis. While the importance of this factor cannot be denied, the concept that alcohol exerts no deleterious effect on the liver, other than by reducing the dietary intake, may be challenged on the basis of both experimental and clinical evidence. Under carefully controlled dietary conditions, Lowry and his associates[17] showed that alcohol increased the severity of the cirrhosis produced by deficient diets in pair-fed rats. Similarly, the hepatotoxic effects of chloro-

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form[25] and carbon tetrachloride[16] could be augmented by alcohol. Clinical observations suggest that alcohol may have a similar action in man. For example, dietary deficiency appears to result in fatty cirrhosis much more frequently in chronic alcoholism than in any of the wasting diseases,[14] or in the uncomplicated malnutrition of prisoners-of-war,[26] and the withdrawal of alcohol frequently results in marked improvement in the histological and functional status of the liver in chronic alcoholics with cirrhosis, even when no dietary suppements are provided.[15]

Such observations suggest that alcohol may exert an influence on the liver, aside from its effects on the dietary intake, either directly by some toxic or metabolic action, or indirectly by increasing the nutritional requirements of the liver. In 1947 Ashworth[1] reinvestigated this aspect of the problem and concluded that alcohol exerted a direct effect on the liver cell, permitting fat accumulation unrelated to an extrinsic deficiency of lipotropic factors. He found that rats on a high protein diet (25 per cent casein), given the equivalent of 0.6 ml. of absolute ethyl alcohol per 100 grams of body weight daily by stomach tube and in drinking water, developed fatty livers at the end of two months, while pair-fed controls receiving no alcohol did not.

In pair-feeding his animals Ashworth[1] did not take into account the caloric value of alcohol, so that the total caloric intake differed in the two groups (Fig. 1). Moreover, the vitamin supplement was inadequate in some respects, and the method of administering alcohol resulted in intoxication and periods of coma, leading to marked restriction of food intake and significant weight loss, especially in the non-alcohol group. It is possible, therefore, that the fatty infiltration in the alcohol group was related to the increased caloric intake, or conversely, that fat failed to appear in the non-alcoholic group because of the more severe weight loss.

In 1949 Best and his associates[4] carried out similar but more carefully controlled experiments and came to the conclusion that alcohol did not exert a specific toxic action on the liver. These investigators not only pair-fed alcoholic and non-alcoholic rats, as in the Ashworth experiment,[1] but also included a control group which was both pair-fed and kept isocaloric by substituting sucrose for alcohol calories (Fig. 1). By administering all their alcohol as a 15 per cent solution in drinking water, and by providing an 18 per cent casein diet adequately supplemented with vitamins, they obtained satisfactory food intakes and good growth in their animals. Under these conditions fatty infiltration and pre-cirrhotic fibrosis of the liver developed in those rats receiving supplements of alcohol or isocaloric equivalents of sucrose for a period of six months. Since these lesions could be prevented by dietary supplements of choline, methionine, or casein, it was concluded that when the diet was marginal with respect to choline, excesses of alcohol or sucrose induced a relative deficiency of choline by increasing the caloric intake.

Although isocaloric equivalents of alcohol and sucrose both produced fatty

infiltration of the liver which could be prevented by lipotropic dietary supplements, the conclusion that both exerted their action by increasing the total caloric intake was not clearly established in these experiments. Thus, it was not shown that the hepatic effects of alcohol could be prevented when the total caloric intake was kept down to that of pair-fed controls by subtracting non-protein calories. Moreover, the effects of alcohol were complicated by a partial deficiency state. Of the 18 per cent protein provided in

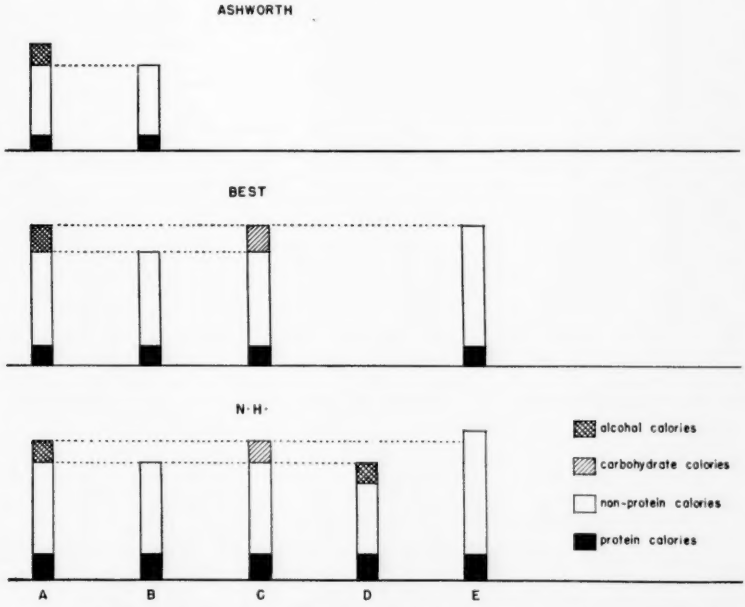

FIG. 1. Schematic comparison of experimental designs employed by Ashworth,[1] Best *et al.*,[4] and the authors (N.H.).

the basal diet, almost half was comprised of gelatin and zein, poor quality proteins deficient in tryptophane, tyrosine, and cystine and unsuited for growth or maintenance.[5] The cystine requirement was met by a suitable supplement, but no provision was made for the other deficits. In addition the diet was slightly deficient in choline and methionine so that even the control group of rats fed *ad libitum* had abnormally high liver lipids (7.7 to 12.8, average 9.3 per cent). Thus, whatever effects alcohol had were engrafted on those of a partial deficiency state, so that alcohol may have merely aggravated the hepatic injury produced by a deficient diet, as in Lowry's experiment.[17]

Since the investigations of Ashworth[1] and Best *et al.*[4] left these questions unanswered, experiments were carried out in rats to determine: (a) whether alcohol produces fatty infiltration and fibrosis of the liver when the diet is adequate with respect to protein, lipotropic factors, and vitamins for *ad libitum*-fed control animals, and (b) whether alcohol increases the requirements for choline by augmenting the caloric content of the diet.

Experimental

Animals. Sixty weanling rats of the Sprague-Dawley strain, weighing approximately 50 grams each, were placed on a vitamin-enriched, synthetic diet known to insure rapid growth and good nutrition. In 20 days, when they had attained a weight of approximately 150 grams, they were transferred to individual cages and started on the experimental regime to be described. The cages were arranged in rows of 12 on a 5-shelf rack in a constant-temperature, air-conditioned room kept at 25°C., and a humidity of 50-55 per cent. Each of the 12 animals in group A on the top shelf had its own 4 control animals belonging to groups B, C, D, and E respectively beneath it. All diet calculations were made on the basis of the food and alcohol intake of the group A animal in each such group of 5. The animals were weighed weekly to the nearest gram.

Feeding technique. The design of the present experiment and, for purposes of comparison, the design of those carried out by Ashworth[1] and Best,[4] are schematically represented in Figure 1.

Group A received the basal diet and alcohol *ad libitum.*

Group B was pair-fed the basal diet with group A, but received no alcohol, so that the two groups were not isocaloric.

Group C was pair-fed the basal diet with group A, and was given an isocaloric equivalent of glucose instead of alcohol, so that both groups were isocaloric.

Group D was pair-fed the basal diet and was given the same amount of alcohol as in group A, but the total caloric intake was not permitted to rise above that of the non-alcohol controls in group B; this was accomplished by subtracting glucose calories from the basal diet isocaloric with the amount of alcohol consumed.

Group E received the basal diet *ad libitum,* without alcohol.

The basal diet was designed to promote normal growth and to maintain a normal fat concentration in the livers of the control animals in group E. Groups A and C were kept isocaloric to determine whether isocaloric equivalents of alcohol and glucose would produce equal fatty infiltration under these conditions. Group D was given an amount of alcohol equal to that consumed by group A, but was kept isocaloric with group B, to test the hypothesis of Best *et al.*,[4] that alcohol creates a relative choline deficiency by increasing the caloric intake. If such were the case, group D rats receiving alcohol would show no more liver fat than group B receiving no alcohol.

The diet for group A was weighed out in tared feeding cups at three-day intervals. At the end of each period the cups were reweighed and the diet consumed calculated by difference. The amount of food for groups B, C, and D was then calculated from this amount as outlined below. As a rule, the rats in group B, C, and D ate all the food that was offered, but if they did not, the residue was mixed with the new diet. Occasionally during a temporary illness when the food intake fell off markedly, the residue was discarded because it continued to increase cumulatively beyond the animal's feeding capacity. Such divergences from the plan of the experiment are included in the calculated data of Table 1. Animals in group E were fed *ad libitum* out of tared cups in a similar manner.

The *basal diet* had the following composition:

	Per cent	*Calories*	*Per cent calories*
Vitaminized casein*	10	88	17.7
Labco casein	12		
Vitaminized corn oil*	5	225	45.3
Lard	20		
Glucose	46	184	37.0
Salts IV	5		
Agar	2		
	100	497	100

* Vitamins (per 100 grams of diet)

B_1, 1 mg.
B_2, 1 mg.
B_6, 1 mg.
Nicotinic acid, 10 mg.
Pantothenic acid, 10 mg.
Biotin, 100 gamma.
Folic acid, 100 gamma.
Inositol, 20 mg.
Paraminobenzoic acid, 20 mg.
Alpha-tocopherol acetate, 10 mg.
Vitamin K, 500 gamma.
Vitamin D_3, 10 gamma.
Vitamin A, 3000 I.U.

In group C the calculated glucose supplement, isocaloric with the alcohol intake of its pair-fed mate in group A, was mixed with its three-day food allotment.

In group D the rats received an amount of alcohol equivalent to that taken by group A for the preceding three-day period, but an isocaloric quantity of carbohydrate was subtracted from the basal diet to keep the total caloric intake down to that of group B. For the sake of convenience and ease of calculation, a *carbohydrate-free diet* was prepared which had a composition identical with that of the basal diet except for the absence of glucose:

	Grams	*Per cent*	*Calories*	*Per cent calories*
Casein	22	40.7	162.8	28
Oil and lard	25	46.3	416.7	72
Agar	2	3.7		
Salt	5	9.3		
	54	100	589.5	100

Since the carbohydrate-free fraction of the basal diet constituted 54 per cent by weight of the total, rats in group D were given the carbohydrate-free diet in amounts equivalent to 54 per cent by weight of the basal diet eaten by their respective pair-fed mates in group A for the preceding three-day period. The total glucose requirement was then calculated on the basis of 46 per cent by weight of the same diet minus 0.2 gram of glucose for each ml. of 15 per cent alcohol consumed by the pair-fed mate in group A.

Alcohol feeding. Preliminary experiments carried out in this laboratory revealed that animals fed alcohol by gavage in amounts equivalent to 0.6 gram of absolute ethyl alcohol per 100 grams of body weight per day were comatose for long periods, ate little, lost weight rapidly, and often succumbed. It was deemed necessary, therefore, to administer alcohol as a 15 per cent solution by volume in drinking water. Various types of drinking bottles were investigated, including that described by Best *et al.*,[4] but marked losses by leakage could not be eliminated. A search of the literature called our attention to the water fountain designed by Richter.[24] This proved to be highly

satisfactory for our purposes, since no losses by leakage or splashing occurred in any of our preliminary experiments, and the loss by evaporation was found to be negligible (less than 0.3 cc. of 15 per cent alcohol per day). The water fountain was filled and then fixed to one corner of the cage with a metal clamp.

Groups A and D received a 15 per cent dilution by volume of 95 per cent ethyl alcohol as their only source of drinking water; 1 ml. of this solution was equivalent to 0.113 gram of absolute alcohol and had a caloric value of 0.79 Calories. In calculating the isocaloric quantity of glucose to be added in group C, and subtracted in group D, alcohol was given its full caloric value of 7 Calories per gram, so that each ml. of alcoholic drinking water consumed was equivalent to 0.2 gram of glucose. In the Toronto experiments[4] alcohol was given a value only three-quarters of this amount, on the basis of Mitchell's experiments[20] showing that the energy of alcohol was not as well utilized for physiological purposes as that of sucrose, insofar as its growth-promoting powers was concerned. However, since the energy of alcohol is fully released following absorption, even if only for the non-functional expenditure of heat, the use of this correction factor did not appear to be warranted.

Alcoholic drinking water was fed *ad libitum* in group A, and the amount consumed determined by measuring the decrease in volume of fluid in the alcohol-fountains at three-day intervals. Equivalent volumes were then introduced into the fountains of properly paired rats of group D. As a rule, the animals in group D consumed all the alcohol offered, but when they did not, the residue was added to their next three-day requirement. If during temporary illness there was a marked fall in intake and a progressive increase in the residues, the latter were discarded. Such deviations from the plan of the experiment are incorporated in the data in Table 1.

Chemical and histological methods. One-half the animals were sacrificed by decapitation at 117 days, the remainder at 180 days. The liver was dissected free, dried by blotting, and weighed. Wedges of tissue for histological examination were then excised from the right and left lobes and fixed in 10 per cent formalin and in Carnoy's solution. Sections of the formalin-fixed block were stained with osmic acid and Sudan IV, while the Carnoy's-fixed block was imbedded in paraffin and stained with hematoxylin and eosin and a modification of the Masson trichrome stain.

The remaining liver was reweighed and dried overnight in an air-oven at 100° C. Total lipid analyses were then carried out by the A.O.A.C. method.[2]

Results

Growth curves. The diets employed supported good growth in both alcohol- and non-alcohol-fed groups (Fig. 2), and except for transient illnesses and one death (rat 2D), the rats remained in excellent health. Group E receiving the basal diet *ad libitum* gained weight more rapidly than group A on the same diet supplemented with 15 per cent alcoholic drinking water (Fig. 2). This difference was due to a reduction in food consumption related to the ingestion of alcohol and a lower total caloric intake, including that contributed by alcohol (Table 1).

The intake of food and alcohol was reasonably constant in group A for a period of three months, when unaccountably on about the 100th day there was a precipitous fall in the consumption of alcohol, and shortly thereafter, of food (Fig. 3), accompanied by a somewhat less striking decline in weight (Fig. 2). Best and his associates[4] observed a similar decrease in weight

TABLE 1

SUMMARY OF MEAN DAILY INTAKE OF DIET AND ALCOHOL

Group	Diet*		15 per cent alcohol		Glucose		Total intake per rat			Intake per 100 gm.† rat		
	gm.	Calories	ml.	Calories	gm.	Calories	Calories	Protein gm.	Alcohol gm.	Calories	Protein mg.	Alcohol mg.
Total Group												
A	10.65±0.83	52.91±4.35	14.72±1.99	11.63±1.56			64.54±5.05	2.34±0.21	1.66±0.25	18.73±1.26	678±58	482±67
B	10.59±0.81	52.64±3.88					52.64±3.88	2.33±0.18		16.10±1.15	712±55	
C	10.44±0.99	51.89±4.91			2.85±0.34	11.40±1.36	63.12±5.37	2.30±0.22		18.41±1.29	670±45	
D	5.90±0.42	34.19±2.43	13.91±0.66	10.99±0.52	2.12±0.41	8.48±1.64	53.67±3.18	2.40±0.70	1.57±0.08	18.40±1.49	824±78	538±51
E	14.80±1.35	73.55±6.71					73.55±6.71	3.26±0.30		18.75±1.71	831±22	
Sacrificed at 117 days												
A	10.69±0.69	53.10±3.81	14.65±2.64	11.58±2.04			64.68±4.90	2.35±0.14	1.66±0.27	19.75±1.01	717±42	503±77
B	11.07±0.61	55.01±3.12					55.01±3.12	2.44±0.13		17.19±0.41	760±40	
C	10.85±0.58	53.92±2.88			2.92±0.41	11.68±1.64	65.26±3.33	2.39±0.13		19.60±0.59	717±10	
D	6.04±0.30	35.00±1.74	13.91±0.70	10.99±0.55	2.18±0.49	8.72±1.96	54.70±2.90	2.46±0.12	1.57±0.08	19.41±0.90	873±59	558±55
E	14.92±1.23	74.15±6.11					74.15±6.11	3.28±0.27		20.00±0.62	886±11	
Sacrificed at 180 days												
A	10.61±0.95	52.72±4.83	14.79±0.98	11.69±0.68			64.40±5.21	2.33±0.25	1.67±0.13	17.71±0.24	640±27	461±49
B	10.12±0.53	50.28±2.89					50.28±2.89	2.23±0.12		15.01±0.55	663±35	
C	10.03±1.14	49.85±5.67			2.78±0.24	11.12±0.88	60.98±6.12	2.20±0.25		17.21±0.72	622±35	
D‡	5.74±0.36	33.26±2.09	13.91±0.63	10.99±0.50	2.04±0.28	8.16±1.12	52.43±3.14	2.34±0.15	1.57± .07	17.20±0.90	766±41	514±31
E	14.67±1.23	72.91±6.11					72.91±6.11	3.23±0.27		17.50±1.54	776±60	

* Basal diet for groups A, B, C, and E; carbohydrate-free diet for group D.
† Calculated for each rat using mean weight for entire period of experiment.
‡ One animal died at 54 days and was not included in the calculations.
± Values = standard deviation.

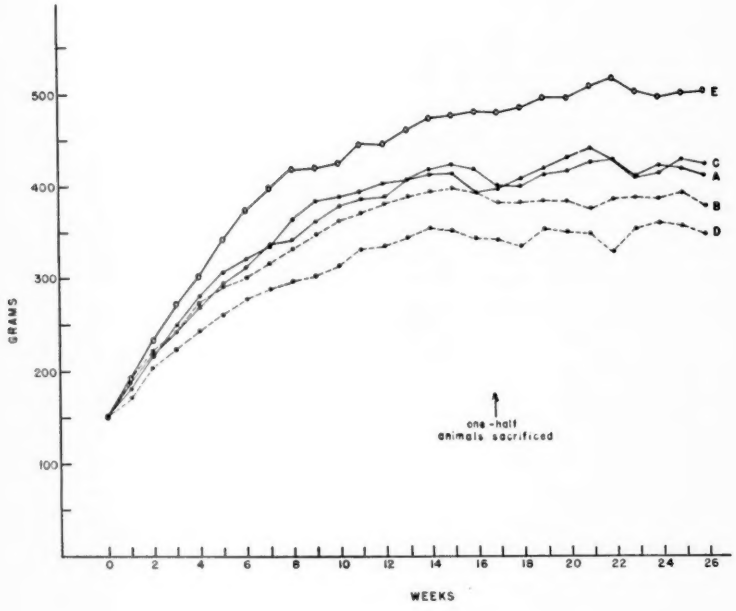

FIG. 2. Growth curves.

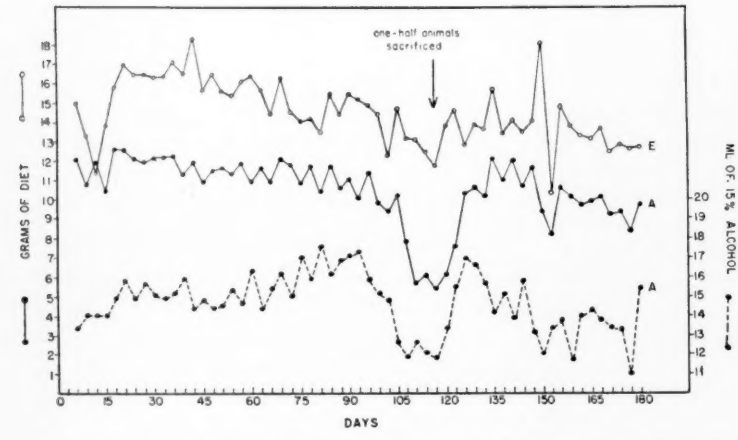

FIG. 3. Food and alcohol intake in Group A compared with the food intake in Group E.

between the 80th and 90th days which they attributed to a period of hot weather, during which there was a failure of their air-conditioning equipment. In the present instance, however, the decrease in food and alcohol consumption occurred at the end of April and did not appear to be related to environmental factors, especially since no similar change in dietary intake or weight occurred in group E maintained under the same conditions save for the absence of alcohol in its drinking water (Figs. 2 and 3).

Six of the 12 rats in group A exhibiting the greatest decreases in food and alcohol consumption were sacrificed along with their corresponding pair-fed mates in groups B, C, D, and E on the 117th day. This was done to avoid complicating the effects of alcohol on the liver by those of malnutrition. The remaining rats rapidly resumed their former eating habits and

TABLE 2

MAXIMUM WEIGHT GAINED BY 100TH DAY OF EXPERIMENT

Group	*Range*	*Mean*	*S.D.*
	gm.	*gm.*	*gm.*
A	133 — 341	262	± 56
B	203 — 291	241	± 26
C	188 — 308	266	± 37
D	172 — 232	205	± 17
E	138 — 406	321	± 69

Mean of group E significantly greater than that of all other groups (smallest difference between means in groups C and E; $t = 2.43$, $p < .05$).
No significant difference between means in groups A and C ($t = 0.1$, $p > 0.9$).
Mean of group B significantly greater than that of group D ($t = 4.02$, $p < .01$).

their former rate of growth, but their alcohol consumption was erratic (Figs. 2 and 3). Nevertheless, the mean daily consumption of food and alcohol in the animals sacrificed on the 117th and 180th days respectively did not differ significantly (Table 1).

The relative nutritive value for growth of isocaloric equivalents of alcohol and glucose was studied by comparing the maximum gain in weight attained by the 100th day in groups A and C and in groups B and D (Table 2). Group A rats fed the basal diet *ad libitum* plus a supplement of alcohol grew as well as group C rats pair-fed the same diet plus an isocaloric supplement of glucose, suggesting that under these conditions alcohol was as well utilized as glucose in promoting growth. However, group B, fed the basal diet alone, gained more weight than group D which received the same number of calories but with an isocaloric substitution of alcohol for part of its glucose ration. Under these conditions alcohol appeared to be less effectively utilized in promoting growth, as suggested by Mitchell.[20] It is difficult to interpret this apparent discrepancy in the relative growth-promoting effectiveness of alcohol and glucose under these two conditions. However, Mitchell[20] pointed

out that the energy of alcohol was less available for growth than that of sugar under the special conditions of his experiment, but that there were many factors, such as the degree of motor activity, the environmental temperature and changes in food absorption, which might obscure this difference under other conditions. In the present experiments these factors were not studied, so that no conclusions regarding the utilization of alcohol cal-

TABLE 3

COMPARISON OF DAILY FOOD AND ALCOHOL INTAKE IN TORONTO[4] AND PRESENT (N.H.) EXPERIMENTS

	Basal diet + alc. ad libitum		*Pair-fed basal diet*		*Pair-fed basal diet + carbohydrate*		*Basal diet* ad libitum	
	Toronto	*N.H.*	*Toronto*	*N.H.*	*Toronto*	*N.H.*	*Toronto*	*N.H.*
Initial weight, gm.	160*	152	160*	153	160*	153	160*	153
Maximum gain, 180 d., gm.	182	293	137	265	198	289	262	370
Mean weight	275*	363	256*	340	304*	360	?	419
Diet consumed, gm.	11.4	10.7	11.2	10.6	11.2	10.4	14.9	14.8
Diet, Calories	50	52.9	49	52.6	49	51.9	65	73.6
Alcohol, 15 per cent, ml.	18.3	14.7						
Alcohol, abs., gm.	2.07	1.66						
Alcohol, Calories	14.5**	11.6						
Extra carbohydrate, gm.					2.7	2.9		
Extra carbohydrate, cal.					10.8	11.4		
Total Calories	64.5	64.5	49	52.6	59.8	63.3	65	73.6
Protein, gm.	2.09	2.34	2.05	2.33	2.05	2.30	2.73	3.26
Methionine, mg.	48	70	47	70	47	69	63	
Choline, mg.	5.7	—	5.6		5.6		7.5	98
Per 100 gm. rat								
Calories	23.5	18.7	19.1	16.1	19.7	18.4	?	18.8
Protein, mg.	760	678	801	712	659	670	?	831
Methionine, mg.	17.5	20.3	18.4	21.4	15.5	20.1	?	24.9
Choline, mg.	2.1		2.2		1.8		?	

* Estimated from Chart 1 in Best *et al.*[4]
** Recalculated on basis of 7 Calories per gram of absolute ethyl alcohol.

ories are warranted. However, it may be stated that, under some conditions at least, alcohol is as well utilized as sugar, and that in any event the full energy of alcohol is exerted even if only in heat production, so that the method of substituting glucose for alcohol in the present experiment, on the basis of a 4 to 7 caloric ratio, was justified.

The rate of growth in groups A, B, C, and E was considerably greater than that in comparable groups studied by Best *et al.*[4] for a similar period of time. On the average, the former gained from 41 to 91 per cent more weight than the latter (Table 3). This could not be attributed to a difference

in the intake of food or of calories, since the latter did not vary by more than 13 per cent in the two groups, nor could it be related to differences in alcohol consumption since the same differential in weight gain was noted in the groups receiving no alcohol. The most significant differences between the groups were in the carbohydrate to fat ratios and in the protein content of their respective diets. It is highly probable that the slower rate of growth in the Best experiment was due to the somewhat lower protein content of the diet (18.3 as compared to 22 per cent), and the inclusion of a high proportion (44 per cent) of poor quality protein in the form of gelatin and zein. It is difficult to compare either of these experiments with those of Ashworth,[1] since the actual food intakes of his animals were not included in his paper. However, it is noteworthy that on a 25 per cent casein diet plus alcohol, his animals lost 10 per cent of their initial body weight in 50 days,* while group A in the present experiment gained 113 per cent during a similar period. Likewise, there was a loss of 25 per cent in body weight in 60 days* in animals pair-fed the same diet without alcohol, while comparable animals in group B in the present experiment gained 111 per cent. These marked differences were probably more closely related to poor food consumption incidental to alcoholic intoxication than to any deficiency in the diet used by Ashworth, although the vitamin supplement was inadequate in many respects.

The calculated alcohol consumption in the Toronto experiments[4] was 25 per cent greater than that of groups A and D (Table 3). However, the accuracy of the measurements in the former experiment may be questioned in view of the type of alcohol feeder employed. These investigators used an inverted bottle and a curved glass delivery tube with a constricted tip. In this laboratory there were considerable and unpredictable losses due to leakage with this type of apparatus. Evidently the Toronto workers encountered similar difficulties, since they were obliged to discount unusually large intakes as probably indicating undetected leakage. It is noteworthy that of the 12 rats in group A fed the same type of 15 per cent alcoholic drinking water *ad libitum*, but by means of a Richter water fountain[24] which permits accurate measurements of fluid intake, only 5 had an occasional daily intake exceeding 18 ml., while the Toronto investigators reported a mean intake of 18 ml. for their entire group.

Liver lipids (Table 4). The mean total lipid concentration in the livers of group E fed the basal diet *ad libitum* was 5.3±1.3 per cent of the wet weight, and in only one instance did it exceed 7.0 per cent. The mean concentrations in all the other groups studied were also within normal limits, but the range and distribution of values in both alcohol-fed groups differed significantly from those in non-alcoholic groups B, C, and E. Thus, all 3 concentrations above 10 per cent and 7 of the 11 concentrations between 7

* Estimated from growth curves in Ashworth's paper.[1]

and 10 per cent occurred in groups A and D fed alcohol, while 32 of the 45 values below 7.0 per cent occurred in groups B, C, and E. Moreover, the mean concentration of lipid in group D was significantly greater than in non-alcoholic, isocalorically-fed group B, and *ad libitum*-fed group E. While the mean fat content of the liver in the alcohol-fed rats of group A also exceeded those of non-alcoholic pair-fed, isocalorically-fed and *ad libitum*-fed animals in groups B, C, and E, respectively, the differences were not statistically significant. However, when the results of the lipid analyses in the groups sacrificed at 117 days were considered separately, it was found that the mean fat content of the livers in both alcohol-fed groups were significant-

TABLE 4

TOTAL FAT CONTENT OF LIVER EXPRESSED AS PER CENT OF FRESH (WET) WEIGHT

Group	*All animals*		*Sacrificed at 117 days*		*Sacrificed at 180 days*	
	Range+	*Mean**	*Range*	*Mean*†	*Range*	*Mean*‡
A	2.0–13.0	6.1±2.8	5.0–13.0	7.8±2.8	2.0–6.0	4.4±1.4
B	3.2– 7.1	4.9±1.0	3.2– 5.8	4.3±0.9	4.6–7.1	5.5±0.8
C	2.1– 8.0	5.0±1.8	2.1– 8.0	4.6±2.2	3.4–6.8	5.4±1.1
D	3.9–16.6	8.1±3.3	8.0–16.6	10.2±3.1	3.9–7.7	5.6±1.3
E	2.8– 7.2	5.3±1.3	4.0– 6.7	5.6±1.0	2.8–7.2	5.0±1.4

± values = standard deviation

* D significantly greater than B ($t = 3.02$, $p < .01$) and E ($t = 2.59$, $p < .02$); other differences between means not statistically significant.

† A significantly greater than B ($t = 2.69$, $p < .05$) and C ($t = 2.60$, $p < .05$); D significantly greater than B ($t = 3.78$, $p < .01$); and E ($t = 2.94$, $p < .02$); other differences between means not statistically significant.

‡ Differences between means not statistically significant.

\+ The frequency distribution of values in groups A and D, based on three groupings (> 10.0, 7.0–10.0 and < 7.0), differed significantly from that of groups B, C, and E (Chi-square = 9.42, $p < .01$).

ly greater than in group E, that the concentration in group A exceeded that in pair-fed group B and isocalorically-fed group C, and that the concentration in group D exceeded that of isocalorically-fed group B. The lipid level in group D was also higher than in group A, but the difference was not statistically significant.

None of the differences between alcohol and non-alcohol-fed rats was observed in the groups sacrificed at 180 days. In searching for factors to account for the discrepancy between the results at 117 and 180 days, no significant differences in the mean daily intakes of alcohol, protein or calories were found. Moreover, the mean alcohol intake between the 117th and 180th days did not differ significantly from that preceding it, so that it was unlikely that recovery from the effects of alcohol had taken place during this period; nor could significant correlations be demonstrated between the individual liver lipid values and the mean daily intake of alcohol, protein, or calories. It is highly probable, therefore, that those animals whose food and

water intakes were most seriously impaired by alcohol, and who were, therefore, killed at 117 days, also had livers which were most susceptible to the effects of alcohol.

Histological findings. The livers in all five groups studied appeared normal on gross inspection, and hematoxylin and eosin and Masson stained sections examined microscopically revealed normal parenchymal cells without any alterations in lobular architecture, cellular infiltration, or connective tissue proliferation. An occasional large fat droplet was seen in some of these sections, and with about equal frequency in all five groups. Sections stained for fat with Sudan IV and with osmic acid showed somewhat more fat, usually in the form of small, centrally located droplets, graded ± to 1+ in degree. These occurred to some extent in all sections examined. Three sections each in groups A and D, and one each in groups B, C, and E showed 2+ fatty infiltration with small and occasional large fat droplets confined to the central zones of the lobules. Although the three livers with a fat content exceeding 10 per cent all showed 2+ fatty infiltration, there was a poor correlation between the histological findings and the results of the chemical analyses in the others.

Discussion

The results of these experiments differed from those of Ashworth[3] and Best *et al.*,[4] in that the ingestion of alcohol did not lead to significant histologically demonstrable fatty infiltration or cirrhosis of the liver. However, on chemical analysis the fat content of the liver was higher in alcohol-fed rats than in pair-fed or isocalorically-fed controls, suggesting that alcohol had an effect on the liver even under conditions of an adequate dietary intake.

With respect to the failure of the alcohol-fed rats in group A to develop significant histologically demonstrable fatty infiltration of the liver, the question may be raised whether the diet used contained such an excess of methionine that the effects of alcohol were obscured. In the Toronto experiments[4] the basal diet was designed to avoid this complication, by reducing the choline and methionine content to the point where the livers of *ad libitum*-fed controls contained 10 per cent fat, a relatively high value when compared with the 5.3 per cent level of the controls in this experiment (group E). The estimated daily methionine intake in group A was 70 mg., in contrast to 48 mg. in similarly fed rats in the Toronto experiment (Table 3). However, in the latter case the methionine was supplemented with 5.7 mg. of choline, and the difference between the intakes was even less significant when they were calculated on the basis of rat weight. Thus, the Toronto animals received 17.3 mg. of methionine and 2.1 mg. of choline, while those in New Haven received 20.3 mg. of methionine alone per 100 gm. of rat daily. Moreover, it took a supplement of 57 mg., or a total intake of 105 mg. of methionine plus 5.7 mg. of choline daily, to keep the total liver lipids

down to 7.0 per cent in the Toronto rats fed the basal diet and alcohol. This is an amount considerably in excess of the 70 mg. of methionine it took to keep the level down to 6.1 per cent in comparable animals in the present experiment (group A). In addition, the choline requirement in the present experiment was probably greater than that in the Toronto experiment, since the fat content of the diet was more than twice as large. It seems unlikely, therefore, that the failure of alcohol to produce histologically demonstrable fatty infiltration in group A was the result of any great excess of methionine. The possibility that it was the result of a smaller alcohol intake cannot be excluded, but is unlikely, as already indicated, in view of the method of alcohol administration employed by the Toronto investigators. Rather, the results suggest that in the Toronto experiments gross fatty infiltration was the result of a partial protein deficiency aggravated by the effects of alcohol.

The conclusion that alcohol induces a relative choline deficiency by increasing the caloric intake, based on the observation that isocaloric supplements of alcohol and sucrose produce similar increases in liver fat and even cirrhosis, and that these effects can be prevented by supplements of choline or methionine,[4] is not supported by the results of the present experiment. In the Toronto experiments sucrose produced even more impressive fatty infiltration and cirrhosis than isocaloric alcohol supplements in pair-fed rats. The fact that these changes could be prevented by lipotropic substances in both instances does not necessarily imply that the effects of alcohol and sucrose were identical. These results do indeed suggest that the addition of sucrose calories increased the choline requirement and thus aggravated the effects of a partially deficient diet. However, the fact that alcohol increased the fat content of the liver in the present experiments, even when the total caloric intake was not permitted to rise above that of pair-fed controls, is strong evidence against the hypothesis that alcohol, like carbohydrate, increases the demand for choline by raising the caloric intake. Rather these experiments suggest that alcohol may increase the choline requirement in some other way, possibly by blocking its action in the liver, by altering its absorption from the intestinal tract, or by altering its rate of endogenous synthesis. Further experiments are needed to test these possibilities.

Summary and conclusions

1. The administration of 15 per cent alcohol as the sole source of drinking water to rats for a period of six months did not produce histological evidence of significant fatty infiltration or cirrhosis of the liver when the diet contained protein, calories, and vitamins sufficient to prevent fatty infiltration in *ad libitum*-fed controls. However, on chemical analysis the fat content of the liver in these rats, while still within the normal range, was greater than in *ad libitum*-fed, pair-fed, or isocalorically-fed controls receiving no alcohol.

2. These results are consistent with the hypothesis that under some conditions alcohol may lead to fatty infiltration of the liver by increasing

the choline requirement, but they do not support the view that this increase is attributable to augmentation of the caloric intake.

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MANIFESTATIONS OF VITAMIN DEFICIENCY DURING AUREOMYCIN AND CHLORAMPHENICOL THERAPY OF ENDOCARDITIS DUE TO STAPHYLOCOCCUS AUREUS*

Report of a Case

HENRY M. GEWIN AND GEORGE J. FRIOU†

A number of side effects have been noted during aureomycin and chloramphenicol therapy. Harris[2] described the untoward effects in detail and suggested that many of these are caused by an interference with the biochemical processes involved in vitamin B metabolism. In addition to the gastrointestinal symptoms of nausea and/or vomiting, epigastric distress, heartburn, and diarrhea, he noted that the stools often became odorless or nearly so in association with a change in the normal intestinal bacterial flora. Mucous membrane manifestations which closely resembled those of pronounced riboflavin deficiency were also seen. Orally administered vitamin B complex did not prevent these lesions. Parenteral administration of a potent preparation of vitamin B complex was more effective in preventing and reversing them. Harris considered these lesions to be due to the destruction of the intestinal bacteria necessary for the synthesis or utilization of members of the B complex.

Several of Harris' patients also developed lesions of the skin and mucous membranes of the perineal region. *Candida albicans* was isolated from these areas, and it was suggested that overgrowth of this fungus following the destruction of the normal intestinal bacterial flora by aureomycin or chloramphenicol might lead to the development of virulent strains capable of invading more vital tissues.

Abnormal bleeding tendencies of minor degree consisting of slight vaginal bleeding in three patients, rectal bleeding in another three patients, and subungual hemorrhage in one patient were also noted by Harris. No special studies to determine the mechanism were made, but vitamin K deficiency was suspected in retrospect.

The following case is noteworthy because it not only illustrates many of the side effects previously described, but it also demonstrates the occurrence of a peripheral neuritis, an optic neuritis, and severe gastrointestinal bleeding associated with prothrombin deficiency and roentgenographic changes in the intestinal mucosa.

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Case Report

S. D., a 22-year-old man, was admitted to the New Haven Hospital on November 7, 1949, with the complaints of headache, chills, and fever of two and one-half days' duration. In 1934, at the age of seven, he experienced his first attack of acute rheumatic fever, and during the next eight years he had four recurrences. In 1942, mitral systolic and diastolic murmurs were noted, and a roentgenogram of the chest revealed left auricular enlargement. Except for dyspnea on strenuous exertion he remained well from 1942 until October, 1949, thirty days before admission to the hospital, when a tooth was extracted without prophylactic administration of antibiotic agents. For the next five days the patient suffered with a "head cold," but was then asymptomatic until two and one-half days prior to admission to the hospital. At this time there was the sudden onset of the first of a series of severe shaking chills followed by fever of 103° F. Sulfonamide powders were begun two days before admission, but twenty-four hours later intermittent chills and fever persisted, and a severe generalized headache appeared. During the next twenty-four hours a stiff neck as well as small painful blue areas on the left leg and right hand were noted. A non-productive cough appeared a few hours before admission.

Physical examination. The temperature was 104.5° F., pulse rate 120, respiratory rate 32, and blood pressure 136 mm. systolic and 86 mm. diastolic. The patient was a well-developed and fairly well-nourished young man who appeared acutely ill. The skin was hot, dry, and flushed, and a diffuse erythematous macular rash was present over the trunk and extremities. There were a few slightly enlarged, non-tender, cervical and axillary lymph nodes. The pharynx was diffusely red but without exudate. The teeth and gums showed no evidence of local infection, and the tongue was well papillated. Slight resistance of the neck to full flexion was noted. A few moist fine rales at the lung bases were the only abnormal pulmonary finding. The heart was enlarged to the anterior axillary line with a diffuse heaving impulse; the rhythm was regular; a harsh, long systolic murmur, loudest at the apex, was transmitted throughout the entire left half of the chest; the mitral first sound was accentuated, and one of three observers heard a faint pre-systolic murmur at the apex on admission. Examination of the abdomen was negative, the spleen not being palpable. A tender ecchymosis (1 by 2 cm.) was present on the right hand in the web between the middle and ring fingers. Purpuric areas were noted on the right great toe and the left fifth toe. All reflexes were equal but hypoactive.

Laboratory examinations on admission gave the following results: The red blood cell count was 4,700,000, the hemoglobin content 14.5 gm., the white blood cell count 11,300 with 7% non-segmented neutrophils, and 82% segmented neutrophils. Urinalysis revealed a 1+ reaction for albumin, numerous coarse granular casts, and occasional white and red blood cells. A lumbar puncture showed clear fluid with an initial pressure of 250 mm. of water. The fluid contained 9 lymphocytes per cu.mm. and 32 mgm. of protein/100 cc. Two routine stool examinations for blood and parasites were negative during the first week.

The admission diagnoses were acute bacterial endocarditis with multiple emboli, and rheumatic heart disease with cardiac enlargement, mitral stenosis, and insufficiency.

Course in the hospital. One hour after admission, as soon as three blood cultures were drawn, aqueous penicillin was started, 300,000 units intramuscularly at once, followed by 100,000 units every two hours.

Twenty-four hours later the temperature remained at 105° F., the lungs showed increasing numbers of rales, the initial embolic lesions had become larger and more necrotic, and three new lesions had appeared. Admission blood cultures were reported at this time to show 800 colonies of *Staphylococcus aureus* per cc. Because of the lack of improvement, and the known resistance of many strains of staphylococcus to

penicillin, aureomycin was started in an initial dose of 3.0 gm. followed by 0.25 gm. every two hours.

Forty-eight hours after admission there still had been no improvement, and for that reason the penicillin dosage was increased to 500,000 units every two hours, only to be discontinued 72 hours after admission when the infecting organism was found to grow in 1000 units of penicillin per cc. Other sensitivity reports at this time indicated that the organism was inhibited by 25 micrograms per cc. of chloramphenicol or 32 micrograms per cc. of aureomycin.

Beginning 48 hours after admission a gradual fall in temperature occurred with concurrent improvement in the patient's general condition, until at the end of one week the temperature was 99° F. During the second hospital week the patient's vital signs remained normal and the original embolic phenomena resolved satisfactorily without new ones appearing. However, a low-grade staphylococcemia persisted despite the disappearance of the phenomena of generalized sepsis. Therefore, on November 21 chloramphenicol was started with an initial dose of 3.0 gm. and 0.25 gm. every two hours thereafter. After three days of combined therapy (aureomycin and chloramphenicol, 0.25 gm. of each every two hours) the blood cultures became negative and remained so during the first course of therapy.

On December 2 the patient noted the onset of anorexia, nausea, and vomiting, and on December 7 a watery diarrhea began. These symptoms persisted despite the use of dramamine (150 mgm. daily) until December 11, when antibiotic therapy was stopped after 33 days of aureomycin and 20 days of chloramphenicol. The nausea and vomiting promptly subsided, but the diarrhea persisted for four more days. Blood cultures had been negative for 18 days at the end of the first course of therapy.

The patient remained well and daily blood cultures were negative for four days following cessation of antibiotic therapy. On the fifth day, December 17, a shaking chill and fever of 102° F. occurred, associated with profuse sweating, a severe headache, and prostration. Blood cultures were obtained; and when these were reported positive for *Staphylococcus aureus* 24 hours later, combined therapy was reinstituted with an initial dose of 1.5 gm. of aureomycin and 1.0 gm. of chloramphenicol followed by 0.25 gm. of each every two hours. During the ensuing week the fever and staphylococcemia slowly subsided. From this time on the patient continued to be free from signs of infection and repeated blood cultures remained negative during a 46-day period of combined therapy and a follow-up period of five months.

Even though there was no further evidence of infection, important symptoms occurred during the second course of therapy. On December 18, combined aureomycin and chloramphenicol therapy had been reinstituted. Four days later erythema and excoriation of the skin over the periscrotal area were noted. On December 24 the patient complained of a sore throat which became progressively worse during the next week. On December 31, anorexia, occasional nausea, frequent pyrosis, and daily vomiting appeared and persisted for seven days despite tincture of belladonna 20 minutes before each dose of antibiotic. Two multivitamin capsules, which had been given daily since admission, were stopped at this time because of the gastrointestinal symptoms. Aluminum hydroxide gel, 8 cc. before each dose of antibiotic medication, was given with slow improvement in nausea and vomiting over a four-day period. However, this medication was stopped because our attention was called to a report[a] that it impeded absorption of antibiotics.

On January 5 distinct evidences of vitamin deficiency were noted, consisting of a red smooth tongue, diffuse pharyngeal injection, and desquamating yellowish papules about the nose and ears. In addition, the skin lesions in the groin were noted to be worse. Symmetrical, scaly yellowish areas of dermatitis with fairly well demarcated borders were seen here. Similar lesions had appeared in the axillae.

On January 5 a liquid vitamin preparation* was started. Within three days there was considerable improvement, and within two weeks all skin and mucosal lesions had disappeared except for slight redness at the base of the scrotum. On January 12 a recurrence of nausea and vomiting was noted, and on January 23 the vomitus became black for the first time. At this time the liver was noted to be enlarged to 3 or 4 cm, below the right costal margin. Liver function tests were done and revealed a positive cephalin cholesterol flocculation (1+ in 24 hours and 3+ in 48 hours) and borderline bromsulphthalein retention of 6.3 per cent after 45 minutes (5 mgm. per kg. injected). The serum bilirubin, thymol turbidity and flocculation, and alkaline phosphatase determinations were within normal limits.

During the next two days the patient regurgitated 400 cc. of coffee ground material which gave a 4+ guaiac reaction. He had three large, tarry stools, following which his red blood cell count fell to 2,100,000 with a hemoglobin of 7.0 gm.. Bleeding and clotting times and a platelet count were normal. His prothrombin time was 15 seconds, control 10 seconds (43 per cent of normal). Daily intravenous therapy with vitamin K (50 mgm.) and vitamins B and C† was begun along with a daily intramuscular injection of 15 units of concentrated liver extract. These medications were continued daily for 11 days until antibiotic therapy was stopped. Despite these measures, as well as 1500 cc. of whole blood, he continued to have tarry stools for eight days until January 31, when his prothrombin time was 12 seconds, control 11 seconds (72 per cent of normal).

On January 31 an x-ray examination of the upper gastrointestinal tract showed an atypical small bowel pattern with segmentation of barium and slight coarsening of the mucosal folds in the jejunum. No ulceration could be demonstrated.

On February 4 antibiotic medication was stopped after 46 days of retreatment, 42 days after the last positive blood culture. A total of 247.5 gm. of aureomycin and 190.5 gm. of chloramphenicol had been given during the two courses of therapy. Six days later a barium enema showed a marked loss of the normal haustral markings of the descending colon. A gastric

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* The daily dose contained: vitamin A, 4800 USP units; vitamin D, 1280 USP units; thiamine hydrochloride, 2.4 mgm.; riboflavin, 1.9 mgm.; ascorbic acid, 64 mgm.; and nicotinamide, 16 mgm.

† The daily dose consisted of: thiamine hydrochloride, 40 mgm.; riboflavin, 12 mgm.; niacinamide, 300 mgm.; pyridoxine hydrochloride, 12 mgm.; panthenol, 12 mgm.; and ascorbic acid, 200 mgm.

analysis revealed free hydrochloric acid after histamine, but the amount was not determined.

On February 14 the patient complained of yellow vision, blurring and fading of letters while reading, and spots in front of both eyes. He stated that these visual symptoms, as well as numbness and tingling of his feet, had been slowly progressing for one week. An examination of his eyes revealed the visual acuity to be 20/25 in each eye. There were slight blurring of both optic discs, granular changes of the pigment layers of the retina, and enlarged blind spots bilaterally. Neurological examination was negative except for patchy, ill-defined areas of diminished pain sensation along the dorsal aspect of all toes and the medial portion of the right lower leg.

On February 18 the patient was discharged after 103 days in the hospital. Discharge medication consisted of ferrous sulfate (0.2 gm. three times daily), one multivitamin capsule daily, and vitamin K tablets (5 mgm. daily).

On March 9 he was seen in the outpatient department. His visual symptoms had disappeared, but numbness and tingling of the feet persisted. Pain on defecation and pruritis ani had appeared since discharge. Liver function tests were repeated and found to be normal. Thiamine hydrochloride was prescribed in an oral dose of 15 mgm. three times daily.

On March 25 the patient reported to the emergency room because of pain in his feet. The numbness and tingling had never disappeared, and two days previously he had begun to experience increasingly frequent lancinating pains in one or more toes, usually the large ones, lasting only one to two seconds, but occurring one hundred or more times daily. A complete neurological examination disclosed diminished testicular pain sensation as well as decreased deep pain sensation of the legs. Tactile sensation was diminished below the knees and absent over the toes. Pinprick perception was decreased over the distal two-thirds of both feet, and over the toes stimulation with the pin produced a diffuse burning pain. Position sensation was intact, but vibratory perception was impaired over the legs and ankles. Motor functions and tendon reflexes were normal. Therapy consisted of vitamin B_{12} in doses of 30 micrograms twice weekly. A total of four injections was given. Within three days after the first injection there was moderate symptomatic improvement, but when examined by a neurological consultant on March 30, there was still evidence of a peripheral neuritis with chief involvement of tactile, pain, and vibratory sensations. Progressive improvement continued, so therapy was discontinued after the fourth injection of vitamin B_{12}.

When seen on May 22, 1950, the patient still complained of burning on defecation and itching about the anus, but was otherwise asymptomatic and normally active. Examination disclosed no change in the heart signs from those described on admission to the hospital except that no diastolic murmur could be heard. There was redness about the anus, and a small posterior

anal fissure was present, but otherwise the examination, including a complete neurological evaluation, was negative. No culture of the skin was taken, but a 1% aqueous solution of gentian violet (methylrosaniline chloride) was prescribed for local application to the perianal area, as suggested by Harris,[2] who isolated *Candida albicans* from similar lesions. Prompt subsidence of the pruritis resulted during the next two days.

Comment

During the course of combined aureomycin and chloramphenicol therapy for staphylococcal endocarditis this patient developed a number of disturbing side effects. There were lesions of the tongue and of the skin of the nose, ears, axillae, and perineal region. Severe gastrointestinal symptoms, including nausea, vomiting, diarrhea, and bleeding, occurred. Gastrointestinal roentgenograms revealed an atypical small intestinal pattern with segmentation of barium. There was also noted enlargement of the liver and slight impairment of hepatic function as measured by functional tests. Visual disturbances associated with objective retinal changes and, lastly, a peripheral neuritis of the lower extremities were also noted. Partial clearing of mucosal and skin lesions resulted from relatively small doses of oral vitamins, but neither oral therapy nor 11 days of parenteral vitamin therapy prevented the subsequent development of the visual disorder or the peripheral neuritis.

The conclusion that these disturbances resulted from the use of aureomycin and chloramphenicol appears warranted from the sequence of events and is supported by the reported experiences of Harris.[2] Since both antibiotics were administered concurrently, neither can be held solely responsible.

The mechanism whereby the untoward effects are produced is still obscure. The resemblance of the buccal lesions to those resulting from deficiency of components of the vitamin B complex, and the frequent occurrence of complicating fungus infections of the skin and mucous membranes has already been discussed. Whether these disturbances result from the destruction of the intestinal bacteria essential for vitamin B synthesis or utilization, or whether aureomycin and chloramphenicol exert a competitive blockage of intracellular metabolism, thus producing changes resembling vitamin deficiency, is a point which remains unsettled. The latter possibility is in keeping with accepted theories of the mechanism of action of the antibiotic agents in bacterial metabolism. If this concept is correct, parenteral administration of potent vitamin preparations may prove to lessen the therapeutic effectiveness of the antibiotic agents, as has been suggested by Crocker.[1] In the present state of our knowledge, however, the withholding of vitamins appears unwise.

The peripheral neuritis seen in this case is probably also attributable to deficiency of a member of the vitamin B complex. The apparent lack of response to thiamine and the striking improvement after vitamin B_{12} is noteworthy, but may be more apparent than real. As suggested by our con-

sultant in neurology, Dr. Paul Yakovlev, the appearance of the most severe leg pains at a time when most of the other signs of deficiency had improved may have indicated returning sensory function rather than increasing neuritis.

The hepatic enlargement may have been related to the fatty infiltration of the liver observed by Yesner and Kunkel[4] in biopsy material obtained during antibiotic therapy in several patients.

At no time did the prothrombin level fall sufficiently low to account by itself for the gastrointestinal bleeding. It may well have aggravated bleeding from a superficial ulceration or a linear tear in the gastric mucosa such as may occur after repeated vomiting; or there may have been an unrecognized vitamin C deficiency. The cause of the prothrombin deficiency, again, could be the result of a change in the bacterial flora of the intestine or of interference with cellular utilization of vitamin K.

Finally, the visual symptoms and the retinal changes remain unexplained. The picture did not appear to be simply that of a vitamin A deficiency, especially in view of the absence of corneal abnormalities.

Summary

A case of staphylococcal endocarditis is reported in which combined therapy with aureomycin and chloramphenicol effected a bacteriological cure but was attended by many distressing side reactions. There were mucosal changes in the mouth and gastrointestinal tract, skin lesions, and peripheral neuritis to suggest an induced deficiency of several components of the vitamin B complex, as well as a complicating fungus infection of the skin. Bleeding from the gastrointestinal tract associated with a prothrombin deficiency and visual disturbances associated with retinal changes suggesting a mild optic neuritis also were noted.

The moderate doses of vitamins given this patient were only partially effective in curing the signs of vitamin deficiencies until the antibiotic agents were stopped.

References

1 Crocker, T. M.: Personal communication.

2 Harris, H. J.: Aureomycin and chloramphenicol in brucellosis. J. Am. M. Ass., 1950, *142,* 161.

3 Waistren, B. A. and Huechel, J. S.: Letter to the editor. J. Am. M. Ass., 1949, *141,* 938; Howard, R. W.: Letter to the editor. J. Am. M. Ass., 1949, *141,* 938.

4 Yesner, R. and Kunkel, P.: Preliminary observations on the effect of aureomycin, terramycin, tibione, combined tibione and streptomycin, and chloromycetin on the morphology of the liver in man. Yale J. Biol., 1951, *23.*

YALE UNIVERSITY SCHOOL OF MEDICINE

ALUMNI DAY PROGRAM

Thursday, February 22, 1951

GENERAL INFORMATION

Registration: All Alumni are requested to register upon arrival at the desk in the lobby of the Sterling Hall of Medicine. This desk will be open at 8:30 a.m., and will close at 6:30 p.m.; it will also serve as a general information center.

There is a registration fee of $2.25 to cover miscellaneous costs. In addition, there is a charge of $2.75 for the luncheon, Club Room, Sterling Hall of Medicine.

To facilitate planning, Alumni are requested to register in advance by mail, and to *reserve their luncheon tickets,* by returning the inclosed card. Luncheon tickets will be mailed if check for $5.00 (registration fee plus luncheon ticket) is received in time; otherwise, tickets may be called for at the registration desk. NOTE: Reservations will be held until 10:45 a.m., Alumni Day.

Rooms: If any Alumni wish to spend the night in New Haven, either before or after Alumni Day, accommodations will be secured for them. If hotel space is not available, private homes will be secured.

Hockey Tickets: These will be secured for Alumni who wish them, provided the registration card requesting them is received prior to February 15 with check inclosed.

UNIVERSITY PROGRAM AND EVENTS

12:30 P.M. Luncheon in the University Dining Hall
Presiding: Edwin Foster Blair, Chairman of the Alumni Board
Speaker: President A. Whitney Griswold

3:00 P.M. Play—"The Knights of the Round Table," given by the Drama Department in the University Theater. No charge.

8:30 P.M. Hockey, Varsity vs. Princeton, Arena. Tickets, $2.40 and $1.50.

PROGRAM

8:00 A.M. *Surgical Operative Schedule,* Farnam Operating Rooms
Thoracic Surgery, G. E. Lindskog, M.D., and Staff
Neurosurgery, W. J. German, M.D., and Staff
Cardiovascular Surgery, W. W. L. Glenn, M.D.
Orthopedics, N. M. Shutkin, M.D.

9:30 A.M. *Grand Rounds, Medicine,*
F. G. Blake, M.D., and Staff, Fitkin Amphitheater

10:00 A.M. *Obstetrics,* Conference, "The New Haven Program for Obstetrical Care," J. B. Forman, M.D., 3023 Farnam Memorial Building

10:30 A.M. *Psychiatry,* Case Conference, F. C. Redlich, M.D., Room 306 Institute of Human Relations

11:00 A.M. *A Symposium, Radioactive Isotopes in Diagnosis and Treatment of Disease; Dangers, and Methods of Control,* Gymnasium
Chairman, L. E. Farr, M.D., Chairman of the Medical Department and Physician-in-Chief, Brookhaven National Laboratories, Long Island, New York
Safeguards in the Use of Radioactive Isotopes, F. Hutchinson, Ph.D., Instructor in Radiation Physics
Use of Radioactive Iodine in Diagnosis and Treatment of Thyroid Disease, W. T. Salter, M.D., Chairman, Department of Pharmacology
Radioactive Phosphorous in Clinical Cancer, D. L. Kline, Ph.D., Assistant Professor of Physiological Chemistry
Radioactive Isotopes in Diagnosis of Blood Dyscrasias, F. D. Lawrason, M.D., Instructor in Medicine

1:00 P.M. *Lunch for Alumni,* Club Room, Sterling Hall of Medicine

2:00 P.M. *Meeting of Yale Alumni in Medicine,* Historical Library, Sterling Hall of Medicine
Presiding, Elihu S. Wing, M.D., President, Association of Yale Alumni in Medicine
The Medical Student at Yale, Laurence Harris, President of the Student Council
Report to the Alumni, Dean C. N. H. Long
Alumni Association Business Meeting, Clyde L. Deming, M.D., Chairman, Executive Committee

3:00-5:00 P.M. *Departmental Open House with Informal Discussions and Demonstrations*

5:00 P.M. *Social Hour,* Club Room, Sterling Hall of Medicine. Dramatic Skit, "First Year Impressions of Yale," by Herbert S. Hurwitz, Harry C. Miller, Jacques M. Quen, John K. Rose, and presented by members of the Class of 1954.

DEPARTMENTAL PROGRAMS FOR OPEN HOUSE

Informal Discussions and Demonstrations

3:00-5:00 P.M.

Under each Department heading are listed research activities in progress. Faculty members will be available to discuss their projects and to show Alumni around their laboratories. The departmental exhibits or demonstrations are indicated by an asterisk.

Anatomy: Room C 216 Sterling Hall of Medicine, William U. Gardner, Ph.D., Chairman

*Experimental Ovarian and Testicular Tumorigenesis, W. U. Gardner, Ph.D., C 216 Sterling Hall of Medicine

*Distribution of E.E.G. Pattern in a Random Group of Medical Students, H. C. Browning, Ph.D., C 216 Sterling Hall of Medicine

*Plasma Progesterone Levels during Pregnancy in Women and Rhesus Monkeys, T. R. Forbes, Ph.D., C 216 Sterling Hall of Medicine

*Genetic Mutations and Mosaics following Injection of Methylcholanthrene, L. C. Strong, Ph.D., C 216 Sterling Hall of Medicine

*Neurosecretion in the Hypothalamus of Man and Lower Animals, S. L. Palay, M.D., C 216 Sterling Hall of Medicine

*Regeneration in the Eye, L. S. Stone, Ph.D., C 216 Sterling Hall of Medicine

Internal Medicine: Room 1074 Laboratory for Medicine and Pediatrics, Francis G. Blake, M.D., Chairman

Antibiotic Therapy, F. G. Blake, M.D., 1074 Laboratory for Medicine and Pediatrics

Metabolic Diseases, J. P. Peters, M.D., 2074 Laboratory for Medicine and Pediatrics

Studies in Serological Epidemiology, J. R. Paul, M.D., B 333 Sterling Hall of Medicine

The Circulatory Effects of Coronary Artery Ligation in Dogs, H. L. Dwyer, M.D., 104 Laboratory for Medicine and Pediatrics

Serum Protein and Cytological Studies in Multiple Myeloma, A. J. Erslev, M.D., 2097 Laboratory for Medicine and Pediatrics

Studies on the Renal Excretion of Electrolytes, A. V. Goodyer, M.D., 107 Laboratory for Medicine and Pediatrics

Studies on Pulmonary Circulation and Ventilation, Frank Gray, M.D., 87 Laboratory for Medicine and Pediatrics

Studies on the Multiplication of Viruses, R. H. Green, M.D., 1091 Laboratory for Medicine and Pediatrics

Experiments on Increasing the X-ray Sensitivity of Tumors, J. H. Heller, M.D., 1071 Laboratory for Medicine and Pediatrics

Metabolic and Histological Studies in Liver Disease, G. Klatskin, M.D., 1087 Laboratory for Medicine and Pediatrics

Studies on the Electrolytes in Red Blood Cells, D. M. Kydd, M.D., 2072 Laboratory for Medicine and Pediatrics

Serological Diagnosis of Rheumatic Fever and Allied Diseases, S. Liao, M.D., and Frieda Gray, M.D., B 336 Sterling Hall of Medicine

Hormonal Control of Electrolyte and Water Metabolism, L. C. Welt, M.D., 2071 Laboratory for Medicine and Pediatrics

Electronmicrographs of Virus Infected Tissues, R. H. Green, M.D., 1090 Laboratory for Medicine and Pediatrics

*Electronmicrographs Demonstrating Elementary Bodies in Skin Papillomata, M. J. Strauss, M.D., H. Bunting, M.D., and J. L. Melnick, M.D., 130 Brady Memorial Laboratory

Coxsackie and Poliomyelitis Viruses, J. L. Melnick, M.D. (Prev. Med., B 322 Sterling Hall of Medicine) and E. C. Curnen, M.D. (Pediatrics, 4087 Laboratory for Medicine and Pediatrics)

*Studies and Photographs with the Electronmicroscope, J. L. Melnick, M.D., and M. Rhian, B 340 Sterling Hall of Medicine

Microbiology: Room 315 Brady Memorial Laboratory, Henry P. Treffers, Ph.D., Chairman

Tumors in Chickens and Ducks. Spreading Factor, F. Duran-Reynals, M.D., Room 323 Brady Memorial Laboratory

Dissertation Problem on Irradiation and Photo-reactivation of *Serratia marcescens.* Enclosed Sharples centrifuge for pathogenic organisms, E. Labrum, Room 326 Brady Memorial Laboratory

*Diagnostic Laboratory for Pathogenic Mycology: Exhibit of Pathogenic Fungi, L. D. Haley, Ph.D., Room 324 Brady Memorial Laboratory

*Seminar Room: Exhibit of Ph.D. and M.D. theses. Staff publications, Room 315 Brady Memorial Laboratory

*Demonstration of Bacteriophage Action. Warburg Apparatus for Measurement of Respiration of Microorganisms, P. B. Cowles, Ph.D., Room 320 Brady Memorial Laboratory

(Graduate student laboratory): Centrifuge Equipment. Lyophile Apparatus for Drying Frozen Biological Material, Room 313 Brady Memorial Laboratory

Material on Antibiotic Resistance of Bacteria. Quantitative Immunochemistry, H. P. Treffers, Ph.D., Room 316 Brady Memorial Laboratory

Dissertation Problems on Counting of Virus Particles and Bacterial Nutrition, W. Gaylord and P. G. Griffin, Room 310 Brady Memorial Laboratory

Cultivation of Viruses in Chick Embryo, B. A. Briody, Ph.D., Room 308 Brady Memorial Laboratory

Material on African Sleeping Sickness. Phase Microscope, D. Weinman, M.D., Room 305 Brady Memorial Laboratory

Refrigerated Centrifuge. Ultrasonic Apparatus for Breaking Down Microorganisms. Cold room, Room 303 Brady Memorial Laboratory

Research on Rh Blood Factor. Lyophile Apparatus for Preserving Cultures, M. Moskowitz, Ph.D., Room 301 Brady Memorial Laboratory

Pathology: Room 2034 Farnam Memorial Building, Harry S. N. Greene, M.D., Chairman

*The Pathogenesis of Arterial Disease, L. L. Waters, M.D., W. B. McAllister, M.D., and G. deSuto-Nagy, M.D., Room 225 Lauder Hall

*Hematological Effects of Atomic Bombing, A. A. Liebow, M.D., S. Warren, M.D., and E. DeCoursey, Col., M.C., U.S.A., Room 130 Brady Memorial Laboratory

Experimental Studies of Cancer, H. S. N. Greene, M.D. and Staff, Room 2034 Farnam Memorial Building

Normal and Pathological Intercellular Substances: Mucopolysaccharides; Mineral Deposits, H. Bunting, M.D., Room 38 Lauder Hall

Aspects of Pulmonary Edema, W. Harrison, M.D., and A. A. Liebow, M.D., Room 232 Lauder Hall

Blood Coagulation, J. H. Milstone, M.D., Room 125A Lauder Hall

Histochemistry of Renal Lesions, T. L. Chiffelle, M.D., Room 38 Lauder Hall

Properties of Collagen as Revealed by Electron Microscopy and Other Techniques, W. G. Banfield, M.D., Room 38 Lauder Hall

The Photofluorimetric Determination of Epinephrine, E. Mylon, M.D., Room 219 Brady Memorial Laboratory

Tissue Culture Studies of Neoplasia, W. S. Albrink, M.D., and A. C. Wallace, M.D., Room 128 Brady Memorial Laboratory

Pediatrics: Room 3089 Laboratory for Medicine and Pediatrics, Grover F. Powers, M.D., Chairman

*New Haven School Cardiac Survey, R. Whittemore, M.D., R. W. Coleman, M.D., and D. L. Dunphy, M.D., Trask Memorial Room, 3109 Laboratory for Medicine and Pediatrics

*Pediatric Cardiac Surgery at the New Haven Unit—Report to Date, W. W. L. Glenn, M.D., and R. Whittemore, M.D., Trask Memorial Room, 3109 Laboratory for Medicine and Pediatrics

*An Outbreak of Acute Nephritis in Adolescent Schoolboys, C. H. Kempe, M.D., R. W. Olmsted, M.D., and E. C. Curnen, M.D., Trask Memorial Room, 3109 Laboratory for Medicine and Pediatrics

*Streptococcal Infections in Children over a Ten-Year Period, E. C. Curnen, M.D., and M. D. Fousek, Trask Memorial Room, 3109 Laboratory for Medicine and Pediatrics

*Virus Techniques in Clinical Investigation, E. C. Curnen, M.D., M. O. Godenne, M.D., and W. E. Gustafson, M.D., Trask Memorial Room, 3109 Laboratory for Medicine and Pediatrics

*Occurrence of the Traits for Thalassemia and Sicklemia in a Single Family, D. H. Clement, M.D., Trask Memorial Room, 3109 Laboratory for Medicine and Pediatrics

*Technique of Exchange Transfusion, V. C. Vaughan, III, M.D., Trask Memorial Room, 3109 Laboratory for Medicine and Pediatrics

The faculty members of the Department will be available for discussion of other problems in Pediatrics

Child Study Center: Room 261 Institute of Human Relations, Milton J. E. Senn, M.D., Director

Pharmacology: Room B 205 Sterling Hall of Medicine, W. T. Salter, M.D., Chairman

*The Positive Ionotropic Action of Certain Fractions of Blood Plasma, W. T. Salter, M.D., and N. Giarman, M.D., B 231 Sterling Hall of Medicine

*The Effect of Certain Di-alkyl Substituted Amino Alkyl Bearing Materials and Related Compounds upon Cyclopropane-epinephrine Induced Cardiac Arrythmias, J. M. White, M.D., and N. Giarman, M.D., B 227 Sterling Hall of Medicine

Studies of Thyroid Physiology and the Treatment of Disorders of Thyroid Activity using among other Substances Radioactive Iodine, W. T. Salter, M.D., B 203 Sterling Hall of Medicine

The Influence of Sodium Lactate upon Thiopental Anaesthesia in the Dog, N. Giarman, M.D., B 224 Sterling Hall of Medicine

Studies upon Emesis Induced by Apomorphine and by Copper Sulfate and of Substances which will Inhibit their Reactions, J. M. White, M.D., B 227 Sterling Hall of Medicine

Studies upon the Activity and Reactions of Analgetic Drugs, D. D. Bonnycastle, M.D., B 316 Sterling Hall of Medicine

A Study of the Mechanism of Production of Agene Epilepsy in Dogs, D. D. Bonnycastle, M.D., B 316 Sterling Hall of Medicine

Physiological Chemistry: Room C 107 Sterling Hall of Medicine, Dean C. N. H. Long, Chairman

The Control of Adrenal Cortical Secretion, Dean C. N. H. Long, W. W. Winternitz, M.D., J. C. Opsahl, M.D., and E. G. Fry, M.S., C 118 Sterling Hall of Medicine

The Enzymic Breakdown and Synthesis of Proteins, J. S. Fruton, M.D., R. B. Johnston, M.D., M. E. Jones, C 116 Sterling Hall of Medicine

The Chemistry of Peptides, J. S. Fruton, M.D., H. Kroll, M.D., P. Y. Dowmont, C 116 Sterling Hall of Medicine

The Nitrogen Metabolism of Microorganisms, S. Simmonds, M.D., D. Stone, M.D., J. S. Fruton, M.D., C 116 Sterling Hall of Medicine

Effect of Diet on Vascular Damage in Diabetic Rat, W. A. Krehl, M.D., and R. R. Rodriguez, M.D., B 348 Sterling Hall of Medicine

Effect of Vitamin Deficiencies on Adrenal Gland, W. A. Krehl, M.D., G. R. Cowgill, M.D., R. W. Winters, R. B. Schultz, B 348 Sterling Hall of Medicine

Mechanism of Lactose Synthesis in Mammary Gland, L. Astrachan, G. R. Cowgill, M.D., and W. A. Krehl, M.D., B 348 Sterling Hall of Medicine

Isotopic Studies on Protein Metabolism, H. D. Hoberman, M.D., J. Graff, J. Doolittle, and P. H. Peralta, C 125 Sterling Hall of Medicine

*Transplants of Pituitary Tissue in the Eye of Hypophysectomized Rats, E. G. Fry, M.S., C 118 Sterling Hall of Medicine

*Demonstration of Eosinophile Technique for the Assay of Adrenal Cortical Activity, E. G. Fry, M.S., C 118 Sterling Hall of Medicine

Physiology: Room B 106 Sterling Hall of Medicine, Donald H. Barron, Ph.D., Acting Chairman

Fetal Circulation and Changes Associated with Birth, D. H. Barron, Ph.D., Room B 107 Sterling Hall of Medicine

Determination of pH, D. I. Hitchcock, Ph.D., Room B 150 Sterling Hall of Medicine

The Nervous Mechanisms Governing Eye Movements, R. B. Livingston, M.D., Room B 116 Sterling Hall of Medicine

Electrical Activity of the Brain, H.-T. Chang, Ph.D., Room B 38 Sterling Hall of Medicine

The Brain Tumor Registry, L. Eisenhardt, M.D., Room B 111 Sterling Hall of Medicine

The Peripheral Circulation, H. Lamport, M.D., Room B 130 Sterling Hall of Medicine

Stimulation of Buried Cortex and Subcortical Structure in the Waking Animal, P. D. MacLean, M.D., Room B 123 Sterling Hall of Medicine

Recent Experiments Bearing on the Functions of the Cerebral Cortex, K. H. Pribram, M.D., Room B 114 Sterling Hall of Medicine

Frontal Lobotomy, H. E. Rosvold, Ph.D., Room B 120 Sterling Hall of Medicine

Physiology of the Kidney, W. D. Blake, M.D., Room B 142 Sterling Hall of Medicine

Food Intake and Experimental Obesity, J. R. Brobeck, M.D., Room B 124 Sterling Hall of Medicine

Aeromedical Unit and Decompression Chamber open for inspection, Room B 46 Sterling Hall of Medicine

Psychiatry and Mental Hygiene: Room 305 Institute of Human Relations, Fredrick C. Redlich, M.D., Chairman

2:00 P.M. Contemporary Research in the Department, F. C. Redlich, M.D., and Staff, 306 Institute of Human Relations

Public Health: Room 216 Brady Memorial Laboratory, Ira V. Hiscock, Sc.D., Chairman

Radiology: Room 2003 Clinic Building, Arnold Janzen, M.D., Executive Officer

Surgery: Room 1036 Farnam Memorial Building, Gustaf E. Lindskog, M.D., Chairman

*Segmental Resection for Pulmonary Tuberculosis, G. E. Lindskog, M.D., D. S. Hubbell, M.D., and R. D. Alley, M.D., Farnam Operating Rooms

*Evaluation of Jugular Vein Blood Flow from Cerebrum as Related to Radical Neck Dissection, A. J. Anlyan, M.D., E. E. Cliffton, M.D., and S. P. W. Black, M.D., Surgical Laboratories, 2nd Floor, Farnam Memorial Building

*Brain Angiography Demonstration by Radiology and Neurosurgery, W. J. German, M.D., Department of Radiology

*Evaluation of Serum Tests for Cancer, E. E. Cliffton, M.D., Room 2036 Farnam Memorial Building

*Studies on Beta-Glucuronidase Activity of Serum, A. J. Anlyan, M.D. Room 2037 Farnam Memorial Building

*Surgical Treatment of Congenital Heart Disease, W. W. L. Glenn, M.D., and F. B. Maraist, M.D., Surgical Laboratories, 2nd Floor, Farnam Memorial Building

*Experimental Studies on Control of Proteolytic Activity in Animals, B. F. Rush, M.D., Room 2036 Farnam Memorial Building

*Treatment of Burns: Use of Pyruvic Acid, S. C. Harvey, M.D., and R. Zimmerman, M.D., Room 2029 Farnam Memorial Building

Military Medicine: 321 Congress Avenue, Major William H. Meroney, III, M.D., in Charge

Medical and Historical Library: Frederick G. Kilgour, Librarian
Exhibit: History of Radioactive Isotopes

CLASSES FOR STUDENTS

Alumni are invited to attend any of the following regular classes and conferences for students which will be in session during the day:

Anatomy:

2:00-3:00 P.M.	Endocrinology of Reproduction, Room C 216 Sterling Hall of Medicine
3:00-4:30 P.M.	Laboratory, Room C 310 Sterling Hall of Medicine

Internal Medicine:

12:00 M.-1:00 P.M.	Noon Clinic, Fitkin Amphitheater

Pediatrics:

8:30 A.M.	Teaching Conference with Fourth-Year Students, W. R. Wilson, M.D., Room 3087 Laboratory of Medicine and Pediatrics
9:00 A.M.	Teaching Conference with Third-Year Students, V. C. Vaughan, III, M.D., Room 3108 Laboratory of Medicine and Pediatrics
9:15 A.M.	Ward Rounds, Fitkin III, G. F. Powers, M.D.; Howard II, D. C. Darrow, M.D.

Pharmacology:

1:30 P.M.	Lecture—"The Parasympathomimetics," D. D. Bonnycastle, M.D., B 301 Sterling Hall of Medicine
4:00 P.M.	Tea, B 203-205 Sterling Hall of Medicine
4:30 P.M.	Seminar Speaker: Lawrence Fairhall, M.D., Scientist Director, Industrial Hygiene Field Headquarters, U.S. Public Health Service, B 204-205 Hall of Medicine

Physiology:

8:30-9:30 A.M.	Peripheral Circulation, D. H. Barron, M.D., B 17 Sterling Hall of Medicine
9:30-12:00 M.	Laboratory: Hemorrhage and Transfusion, J. R. Brobeck, M.D., and L. R. V. Kerby, B 18 Sterling Hall of Medicine
	Alveolar Air, D. I. Hitchcock, Ph.D., B 14 Sterling Hall of Medicine
	Blood Volume, W. D. Blake, M.D., B 11 Sterling Hall of Medicine
	Oxygen Consumption, R. B. Livingston, M.D., B 5 Sterling Hall of Medicine

Public Health:

9:00 A.M.-12:00 M.	Health Education Workshop, Mr. Sol Lifson, Public Health Seminar Room
1:00-3:00 P.M.	
1:00-4:00 P.M.	Hospital Administration, Prof. G. S. Buis, 201 Brady Memorial Laboratory
4:00-5:00 P.M.	Medical Jurisprudence: "Toxocological Problems," W. T. Salter, M.D., Brady Auditorium

Radiology:

4:30-5:30 P.M.	Staff X-ray Conference, Arnold Janzen, M.D., and Staff, Room 2003 Clinic Building

EVENTS

YALE MEDICAL SOCIETY

November 8, 1950

OBSERVATION OF MALIGNANT CELLS IN VITRO. By Warfield Monroe Firor, M. D., Associate Professor of Surgery, Johns Hopkins University.

Upon retransplantation of a laboratory-maintained twelve-year-old tissue culture of fibroblasts from rat areolar tissue, a chance observation was that some of the cells matured into the parent type while others spontaneously became malignant sarcomatous cells. The factors responsible for the malignant change are unknown. With the phase and electron microscopes, minute cellular details can be observed and photographed. In contrast to the normal fibroblasts, the malignant mesenchymal cells show few filamentous processes at the cell perimeter. The latter also possess a characteristic juxta-nuclear mass and an increased amount of fat globules. Nuclear changes seem to precede cytoplasmic change in the transformation to the malignant types. In one instance, the simultaneous death of a normal cell in close proximity to a malignant cell undergoing mitosis was recorded on film. It was postulated that this might be a possible mechanism for the invasive properties of malignant tissues.

W. F. S.

ATYPICAL GROWTH RESEARCH UNIT

November 29, 1950

METASTASES IN CANCER. By Dale Rex Coman, Professor of Pathology, University of Pennsylvania School of Medicine.

In cultures of human tissue there appeared to be more free-floating cells in the neighborhood of tumor tissue than around normal tissue. This observation led to experiments which showed that, by two different techniques, tumor cells could be separated from one another more easily than could normal cells. This looseness between tumor cells was said to be "associated with" a low calcium content.

The number of metastases found in laboratory animals varied directly with (1) the number of tumor cells injected, (2) the length of time a transplanted tumor remained in the host, and (3) the initial size of that transplant. The "soil hypothesis" of tumor growth was questioned on the basis of experiments where injection of malignant cells into the femoral vein of dogs led to metastases to leg muscle. Also, it appeared that malignant cells could metastasize from the systemic veins to the bones of the axis, skull, and pelvis—presumably through the vertebral venous plexus.

Finally, animal experiments in which stained cells and viable cells were injected for comparison of their subsequent distribution demonstrated that metastases occur in those tissues where embolic cells reach capillaries, and not where the cells get only as far as the arterioles.

J. F. S.

PHARMACOLOGY SEMINAR

December 14, 1950

RECENT DEVELOPMENTS IN ANTIMICROBIAL THERAPY. By Lawrence B. Hobson, M.D., Associate Medical Director, E. R. Squibb and Sons, New York City.

In clinical studies with the various antitubercular drugs, resistance of *Myco. tuberculosis* to streptomycin has been found to occur within two months if the antibiotic is administered daily. By administering 9 grams of p-amino salicylic acid/day and 1-2 grams of streptomycin every fourth day, the emergence of resistant strains of the tubercle bacillus has been delayed for as long as one year. Toxicity symptoms of PAS—nausea, vomiting, and diarrhea—have been ascribed to impurities in the drug. The sodium salt of PAS may be injected subcutaneously with hyaluronidase or intravenously with heparin in doses as high as 30-40 grams/day without the appearance of toxic symptoms, indicating that the aforementioned toxic symptoms are local effects. Tuberculosis meningitis has been treated recently with intrathecal injections of tuberculin and streptomycin. Although there is a disturbing initial response, favorable results have been reported. Tibione in the treatment of tuberculosis is less effective and more toxic than the initial investigations indicated; neomycin and viomycin both cause 8th nerve damage, the former giving rise to kidney disturbances as well; dihydrostreptomycin produces more auditory damage but less vestibular damage than streptomycin.

J. P. G.

YALE MEDICAL SOCIETY

January 4, 1951

THE CONTROL OF INFECTION IN BURNS. By Leonard Colebrook, formerly Professor of Immunology and Bacteriology, St. Mary's Hospital Medical School and the University of London.

Despite the known considerable benefits (e. g., earlier skin grafting) to be derived from controlling infection in burns, chronic low grade infections appear in almost all burn wounds during their treatment in hospitals at the present time. Dr. Colebrook's thesis is that these chronic infections are maintained largely by aerial cross-infection and can be greatly reduced by changing burn dressings in a special, clean environment—not in dusty open wards.

The degree of bacterial contamination of air and its variation with time can be shown by means of a "slit sampler," an apparatus which draws air through a brass cylinder, directing it outward against a blood agar plate in such a manner that the growth later observed on any section of the plate can be ascribed to the bacterial contamination of the air at a particular moment. With this apparatus, one can appreciate the suddenly raised and often qualitatively unique contamination arising from changing dressings of infected burns. Also, since the level of contamination in an ordinary room falls spontaneously only very slowly, the successive treatment of many infections in the same room—as in an open ward, for example—results in a greatly elevated level of contamination.

Ordinary outdoor air, however, is relatively free of bacteria and so the special burn treatment room that Colebrook and his group are using is simply a room that is continually flushed with clean air from the outside. The air is filtered, heated, humidified, and directed obliquely downward from the ceiling so as to provide a continual descending blanket of clean air which is removed by an exhaust fan at the base of the wall. This is a simple, inexpensive, and—the "slit sampler" has shown—effective measure, for the bacterial contamination in such a room is negligible within half an hour after a patient has been treated, the room then being ready for another

patient. A record of swabs taken from dressings has given clinical confirmation of the success of this procedure in combatting cross-infection. Streptococcal infection, for example, which ordinarily reaches at least 30% has been kept below 5%.

A seeming drawback to this measure is that the average single general hospital does not receive enough cases of severe burns to warrant the recommended preparation. This, however, only points to the need for the establishment of properly prepared "burn centers" which would serve considerably larger communities than does any single hospital.

R. G.

BEAUMONT MEDICAL CLUB

W. H. Carmalt Lecture

January 5, 1951

Almroth Wright, Pioneer in Immunology and the Knowledge of Wound Infection. By Leonard Colebrook, formerly Professor of Immunology and Bacteriology, St. Mary's Hospital Medical School and the University of London.

A tribute to Wright emerged from this discussion of his work by his former colleague. "A great thinker," his influence on the practice of medicine seems to have escaped recognition in the textbooks, although he published some two hundred papers and books. His scientific work falls roughly into three periods. While Professor of Pathology at the Army Medical School in England, he devised methods for the production and standardization of typhoid vaccine, and was largely responsible for successful typhoid vaccination in World War I. During a second period, at St. Mary's Hospital, he studied the opsonic power of the infected patient's serum and in many diseases used vaccine therapy to increase phagocytosis. Finally, his attention was turned to the processes of wound healing, with particular regard to the power of leucocytes to destroy organisms without phagocytosis. In addition to these accomplishments, Wright provided a stimulus for the establishment of bacteriological laboratories in hospitals and contributed numerous clever devices for experimental work. One example is the Wright capsule for the removal of material from wounds.

Comment on his personality revealed him to be somewhat eccentric (in a sense which prompted Cushing to call him "the G.B.S. of Medicine") but a man of exceptional ingenuity, kindliness, and simplicity of purpose. He was the kind of person who injected himself to prove the efficacy of vaccine therapy. His contemporaries knew him as a scientist, an antifeminist, a philosopher, or the central figure of Shaw's *Doctor's Dilemma*. Professor Colebrook would have us remember him as "an intellectual giant" and a "great human being."

O. L. K.

PHARMACOLOGY SEMINAR

January 18, 1951

Adrenergic Blockade and Some of Its Applications. By Henry Haimovici, Assistant Attending Surgeon, Montefiore Hospital, New York City.

In search for chemical substances that will alleviate vasospasm of autonomic origin, four main types of adrenergic blocking agents have evolved —ergot alkaloids, imidazolines, benzodioxanes, and beta-haloalkylamines.

Ergot alkaloids, in contrast to the others, act upon the vasomotor center and vagal nuclei as well as demonstrating a peripheral adrenergic blocking action. Imidazolines, represented by priscoline, cause vasodilatation by both histaminergic activity and adrenergic blockade. Benzodioxanes, or the Fourneau substances, are both sympatholytic and adrenolytic, since they block conduction of impulses as well as the action of epinephrine at the neuroeffector junction. The prototype of beta-haloalkylamines, dibenamine, a substance which blocks the pressor action of epinephrine though not that of angiotonin, reduces both systolic and diastolic pressures in some patients with essential hypertension. The hypotensive action of this drug does not appear until several hours after intravenous administration and its effect persists for 24 to 72 hours. The postural hypotension and tachycardia accompanying dibenamine therapy are minimal with a new analogue of dibenamine, SKF-688F, which can be administered orally. Malignant hypertension does not respond to any of these substances.

In patients with glaucoma refractory to the cholinergic drugs commonly used, miosis, a reduction in ocular tension, and the relief of pain follow from 5 to 6 hours after intravenous administration of dibenamine. The drug acts upon the circular fibres of the ciliary muscle. In addition, dibenamine will abolish hyperhidrosis, and although it is commonly believed that cholinergic fibres alone cause sweating, investigations revealed that epinephrine will induce sweating. Dibenamine blocks only the adrenergic component of sweating.

J. P. G.

BOOK REVIEWS

HUMANISM IN AN AGE OF SCIENCE. Being a Ludwig Mond Lecture delivered at Manchester School of Medicine on 6 October, 1949. By John F. Fulton. New York, Henry Schuman, 1950. 26 pp. $.75.

In this thoughtful little essay, Dr. Fulton expresses his concern over the ever-widening gap between the humanities and the sciences in modern education. Recent great discoveries in science and medicine may result in still more emphasis on the technological, at the expense of the cultural, training of men for the practice of medicine, itself "a great humanistic enterprise." As one means of bridging this gap Dr. Fulton suggests the historical approach—"an approach which draws upon our cultural heritage (the humanities) in giving perspective and meaning to the sciences."

To illustrate his thesis Dr. Fulton cites two historical examples of the interrelation of science and culture. The discovery of the telescope was soon reflected in the prose and poetry of the great masters, John Donne, Ben Jonson, and Milton in particular; and the discovery of the microscope caused even greater stir, as shown by passages from *Hudibras, The Tatler,* and *Gulliver's Travels.*

Years ago Osler, Welch, and others realized the need for liberalizing the education of American physicians, and as a result American medicine profited immeasurably. In these critical times, however, there is a trend, perhaps inevitable, in the opposite direction. Dr. Fulton is to be commended for his efforts to maintain, if not to raise, the standards of the past. Every teacher and student will be richly rewarded for the few minutes it takes to read this brief, delightful, and scholarly essay.

ERNEST CAULFIELD

THE ACTINOMYCETES. By Selman A. Waksman. Waltham, Mass., Chronica Botanica Co., 1950. xviii + 230 pp. $5.00.

Among all the diverse groups of microorganisms, none has presented greater problems in systematic classification than the Actinomycetes. Intermediate in many respects between the bacteria and the true fungi, these organisms have been variously placed and many attempts made to relate them to one or the other group. On the species level, their cultural and perhaps genetic variability has resulted in a multitude of poorly described and little understood species. Admittedly a difficult group to study on morphologic grounds because of their small size, slow and limited growth and adaptive tendencies, previous attempts at classification have been of little value in the identification of particular isolates, yet the very great importance that the group has recently attained as the source of numerous potent antibiotic substances has intensified the need for a usable classification.

Professor Waksman's lifelong interest and familiarity with the group has resulted in the present compilation which in addition to a system of classification contains a wealth of information on the biology of these organisms. Perhaps his most important contribution is the comparison of various previous classifications and the discussion of taxonomic criteria. It is clear that the author does not consider his classification the last word on the group but rather presents it as a basis for more exacting study in terms of modern concepts of genetic and physiologic variation. The generic limits in

Waksman's classification are laid down on a broad morphologic basis, whereas the species determinations are more largely dependent upon physiologic behavior. The erection of the ordinal group, the Actinomycetales intermediate and distinct from the Eubacteriales and the Eumycetes, reflect the author's feeling that this is a heterogeneous assemblage of organisms which are perhaps not too closely related.

The volume contains extensive sections on variation and mutation, metabolism, and enzymatic activity, and the antagonistic effects of Actinomycetes upon other microorganisms. The ecology of the group and their role as plant and animal pathogens are thoroughly discussed, and an essentially complete bibliography of all work on the group presented.

This volume will be invaluable to the microbiologist who wishes to study the group critically or use them in industrial applications, and it will be of more than casual interest to anyone interested in the interrelationships of various groups of microorganisms.

V. M. CUTTER, JR.

CHEMICAL DEVELOPMENTS IN THYROIDOLOGY. By William T. Salter. Springfield, Illinois, Charles C Thomas, 1950. 87 pp. $2.

This small volume, a monograph in the series of *American Lectures in Endocrinology,* provides a useful and stimulating review, documented with 119 references. The principal topics which are developed are discussions of the synthesis of the hormone, its nature as a circulating compound, "blocking" of the gland's activity, and the uses of substances containing radio-active iodine. Particular attention is called to some problems not yet solved by investigators. There is a good index. Dr. Salter is to be commended for an interesting and well-organized brief treatment of an exciting field of research.

T. R. FORBES

GENES, PLANTS, AND PEOPLE. By C. D. Darlington and K. Mather. Philadelphia, The Blakiston Company, 1950. xxi + 187 pp. $4.00.

This small volume is a reprint of eighteen more or less unrelated essays on various genetical subjects published by the authors during the past two decades. The disparate nature of the material covered and the absence of a unifying theme make difficult an overall appraisal. Concerning "genes," the brilliant extensions of cytogenetic theory by the senior author in the first three essays, now over fifteen years old, are perhaps the most satisfying to the critical specialist. However, the distinction between well-established fact and interpretation is not always carefully drawn; this is also true of the provocative discussions of gene action by both authors. "Plants" figure prominently among the examples in all the essays, and Dr. Mather discusses breeding systems in higher and lower plants at some length. The two chapters on "people" concern themselves more with the theoretical extension of population genetics to man than with human genetics as such. After reading Dr. Darlington's essay on *"Race, Class, and Mating in the Evolution of Man,"* one can only agree with his opening remark that "most of the positive opinions that have been advanced owe little to our recent knowledge of heredity." Preserving the language of genetics, he glibly discusses social change in terms of new genetic combinations (Islam) and the utilization of accumulated genetic material (English, French, and Russian revolutions). We should indeed like to know the significance of

such factors in social evolution, but uncritical assertions, especially by so eminent a geneticist, serve only to confuse the issue.

One aim of the authors, as expressed in the preface, is to "introduce the concepts and methods of genetics to the general student." Though the professional geneticist will find, as always in the work of Darlington and Mather, much food for thought, the general student will do well to read with caution.

JOSEPH G. GALL

COLOR ATLAS OF PATHOLOGY. Prepared under the auspices of the U. S. Naval Medical School of the National Naval Medical Center, Bethesda, Maryland. Philadelphia, J. B. Lippincott Company, 1950. xi + 546 pp. $20.00.

Unique in the field of pathology, this publication contains over one thousand superb full color photographs of both microscopic and gross pathologic tissues, each picture complemented by typical clinical histories and brief descriptions of the pathology. All eight sections—hematopoietic, reticulo-endothelial, cardiovascular, musculoskeletal systems, the respiratory and alimentary tracts, the liver, and the kidney and urinary tract—contain an authoritative introduction classifying and discussing diseases affecting that system or organ. Unfortunately, the nervous, endocrine, and genital systems are covered only in part and are distributed sparsely throughout the book. The section on the hematopoietic system is especially noteworthy for its excellent color reproductions of the various blood dyscrasias as seen under the microscope.

So skillful and thorough is the workmanship, so handsome the format, that one is not surprised to learn that six years were consumed in its preparation, a combined effort of top-flight Army pathologists, artists, photographers, color verification specialists, and technicians. Material from the Pathology Department of the Navy's Medical School, the Army's Institute of Pathology, Johns Hopkins Hospital, and Georgetown University Medical School was utilized. Upon the responsible group should be bestowed an accolade for condensing so much information so readably into so few pages. The innumerable problems that beset such an undertaking are immediately evident to the reader. This book is an inspiring example of what can be done when specialists in diverse fields donate their talents.

A practical, succinct, ready reference, filled with visual information, this book will be of use to pathologists, medical practitioners, laboratory diagnosticians, and students. Long a need in the field, this work can be compared only with itself—an exemplary volume which will prove difficult to emulate.

V. S. G.

ADRENAL CORTEX (Transactions of the First Conference. November 21-22, 1949.) Elaine P. Ralli, Ed. New York, Josiah Macy, Jr. Foundation, 1950. 189 pp. $2.00.

Once each year, the Macy Foundation proposes to bring together, for a two-day conference on the adrenal cortex, a group of active investigators in the field who represent different branches of science. In order to permit full participation of all members in the discussion, attendance is limited to a small number, twenty-five or less. Benefits of the conference accrue to a wider group through publication of a verbatim stenographic record of the

proceedings, and the present volume comprises such a report of the first conference, which was held in November 1949, under the chairmanship of C. N. H. Long.

Each of five sessions was opened by a concise presentation of one topic in the field, as follows: Relation of Chemical Structure of Adrenal Cortical Hormones to Biological Activity, by Edward C. Kendall; Regulation of Adrenal Cortical Secretion, by Gregory Pincus; Clinical Studies with Cortisone and ACTH, by Robert F. Loeb; Steroid Metabolism in the Adrenal Cortex, by Konrad Block; and Relation of Vitamins to Adrenal Cortical Function, by Elaine P. Ralli. A lively and extended discussion followed, by no means narrowly confined to the topics introduced. (The additional participants were Walter Bauer, J. W. Conn, E. Gellhorn, Dwight J. Ingle, Robert F. Pitts, George Sayers, Hans Selye, George W. Thorn, Abraham White, and Frank Fremont-Smith, Medical Director of the Foundation.) Undoubtedly the discussants had an opportunity to edit the original transcript, but fortunately for the reader the informal atmosphere of the meeting was not lost in so doing. In most instances the questions considered were clarified. Where information was inadequate to dispel confusion, at least the differing points of view represented at the conference were elaborated, by challenge and rebuttal, beyond that found in more formal publication.

Although many of the opinions expressed, and there were not a few, may otherwise never have reached the printed page, a fair share of the factual material presented has since been published elsewhere in grater detail, and it is regrettable that the report was not published until nine months after the meeting. That, even so, care in proof-reading was slighted in favor of relative promptness of publication is suggested by the occurrence of a rather large number of typographical errors, for example, "desoxycorticosterone" for "17-hydroxydesoxycorticosterone" (pp. 27 and 110), "45-pregnenolone" for "Δ5-pregnenolone" (p. 43), "formaldogenic" for "formaldehydogenic" (p. 135), and "androsterone" for "adrenosterone" (p. 148). Earlier Macy reports, reproduced by a photo-offset process and bound in paper, appeared more promptly, and the modesty of the format did not seriously limit their usefulness.

Despite intensive research on the adrenal cortex now in progress in many laboratories and clinics, the basic questions discussed so incisively at the First Macy Conference still await adequate answers one year later. The discussions which took place at the conference are in almost every instance as pertinent now as they were then, and a careful reading of the report should not be neglected by students of the adrenal cortex. Those less intimately concerned with the subject will find the book instructive, unburdened by preoccupation with technical details, and certainly enjoyable reading.

PAUL L. MUNSON

NATURAL CHILDBIRTH. By Frederick W. Goodrich, Jr. New York, Prentice-Hall, Inc., 1950. 176 pp. $2.95.

This authoritative handbook, designed for the expectant mother, gives important facts about the anatomy and physiology of pregnancy and birth. The three trimesters of pregnancy are discussed separately and a complete course of diet and exercise is given. The chapter entitled "The Physiology of Labor" divides the birth process into stages and describes each stage, along with instructions on breathing, relaxing, and putting

forth effort at the proper times. Immediately following is a chapter entitled "The Psychology of Labor" in which the sensations of the different stages are described, as well as the actual mechanics of the hospital admission and the various procedures usually carried out during labor and delivery. A discussion of breast feeding and of the "rooming-in" plan is included. The concluding chapters deal with the post-partum period and contain suggestions on diet, hygiene, and exercises for the mother.

The subject is treated realistically and practically. The style is informal and the language simple and straightforward. The book is adequately indexed and contains diagrams and anatomical illustrations appropriate to the text.

Many questions which arise during the course of pregnancy are answered in this little book; its principle purpose is to relieve fears and tensions by preparing the mother both mentally and physically for childbirth.

V. S. G.

CHEMISTRY AND BIOLOGY OF PROTEINS. By Felix Haurowitz. New York, Academic Press, Inc., 1950. xii + 374 pp. $5.50.

It appears almost incongruous that such a slender volume should contain so imposing a title, but perusal reveals that almost the entire field of proteins is encompassed—their isolation and purification, physical properties and structure as well as descriptions of enzymes, hormones, immunologic reactions, and protein anabolism. Within the limits set forth by the author in the foreword—"to give a uniform outline of the present state of the protein problem"—the book has fulfilled its purpose. Further, it has filled the long-existing need for a modern single volume correlating information from the medical, chemical, and physical literature; there are over 1,500 original references, some as late as 1950, to the foreign and domestic literature. But the book is no mere recitation of pertinent references; it is well integrated. The subject matter never becomes obfuscated by detail, yet several viewpoints of controversial problems are presented, as exemplified in the discussions of blood clotting and muscle chemistry. Illustrations add to the clarity inherent in the presentation.

The chapters on hormones and enzymes are perhaps too breezy, with oversimplifications, omissions, and errors. For example, the statement (p. 272) that "The hormones of the anterior (pituitary) lobe act very slowly, their effects being manifested only after many hours or days" does not apply to ACTH which can reduce the ascorbic acid content of the adrenal in minutes. More instances of the relation of pK to protein reactions might have been given, a consideration of co-ordination complexes of ions with regard to enzyme action was in order, and the delineation could be made more clearly between (a) apoenzymes that have an inherent catalytic activity which is supplemented by a coenzyme and (b) the converse, coenzymes that are catalytically active—which action the protein facilitates. In the discussion of the contractile process, references to A. V. Hill were notably absent. The purely colloidal aspects of protein chemistry were neglected, and surface chemistry was not adequately discussed. An author index to supplement the excellent subject index would have been profitable, as would a bibliography of general review articles at the end of each chapter. The volume is well edited, and the literary quality commendable.

As a comprehensive review the book meets the needs of graduate and medical students, and can be recommended to research workers or teachers

interested in a rapid, readable survey of the field. For those interested in more limited phases of protein research, the bibliography will prove helpful.

J. P. G.

INTRODUCTION TO THE BACTERIA. By C. E. Clifton. New York, McGraw-Hill Book Co., 1950. 528 pp. $5.00.

The title of this book is well chosen. Dr. Clifton, Professor of Bacteriology at Stanford University, has succeeded in organizing a textbook which gives the uninitiated reader a firm acquaintance with the microbes without forcing him to become familiar with any. A consideration of the contents will reveal the scope of the work. After a brief history of bacteriology, the author defines basic terms and describes microscopy and other bacteriologic techniques, e.g., staining. Then he gives a careful and lucid description of the structure and characteristics of an idealized microorganism, including flagella, cell division, spores, and others.

Next, the author outlines the system of classification—protozoa and algae, fungi, bacteria, and viruses. He has organized much of this section on a phylogenetic basis and occasionally sacrifices simplicity in order to be inclusive. The treatment is broad, however, and is not limited to any one phase of the subject, such as medical microbiology. He then outlines the vital processes of microorganisms—energy requirements, respiration, metabolism, growth, multiplication, and death, in that order. In the last part of the book Dr. Clifton discusses practical aspects of the subject, including soil, air, water, food, and industrial bacteriology. Finally, he turns his attention to medical applications, considering infection and resistance, serology, and infectious diseases. However, realizing that such a long general treatment of the subject would leave a beginner confused, Dr. Clifton has included a chapter on the enteric bacilli in which he elucidates the principles of isolation, identification, and antigenicity, using the members of the group for examples.

Dr. Clifton has succeeded in the difficult task of bringing a measure of order to a subject which, to a beginner, can easily seem chaotic. The organization and scope of his presentation recommend it highly. He has given a very concise and brief bibliography, which is most commendable for an introductory text, since a comprehensive bibliography can discourage and confuse the beginner. Moreover, the author has avoided being dogmatic, indicating clearly that many problems remain unsettled. His style is not marked by the lucid simplicity which a few writers, notably the British, can attain, but it is by no means prohibitive. The book readily recommends itself as a text for college courses and to any reader seeking an introduction to the bacteria.

J. F. S.

THE NATIONAL FORMULARY, NINTH EDITION. The Committee on National Formulary, by authority of the American Pharmaceutical Association. Washington, D. C., Mack Printing Co., 1950. x + 877 pp.

The *National Formulary* (NF) is one of the two drug compendia recognized by Federal law. To the pharmaceutical profession it is the more useful of these. Its ninth edition became "official" on the first of November 1950. A revision by the Committee on National Formulary under the supervision of the Council of the American Pharmaceutical Association is

made each five years. Subsequent changes and corrections are printed in the *Journal of the American Pharmaceutical Association.* Whenever necessary, supplements are issued. The criterion for admission of a drug to the *National Formulary* is its use, whereas criteria for the *United States Pharmacopeia* are indispensibility, uniqueness, and proved worth. The contents of the two books are mutually exclusive except when a drug which is "official" in the *United States Pharmacopeia* may appear in the *National Formulary* as a different salt, in a different dosage form, or as part of a complex mixture.

A total of 162 items were dropped from the *National Formulary* in the preparation of this edition. Some of these deletions are monographs on drugs which are now included in the *United States Pharmacopeia.* Most, however, were dropped because of lack of use. Titles and standards for 155 drugs without other recognition have been added. Formulae and standards for a number of drugs not included in the recent revision of the *Pharmacopeia* are continued.

In addition to the monographs on drugs, chemicals and preparations and the information on history, regulations, revisions, deletions, admissions, and changes, the *National Formulary* contains valuable sections on chemical and physical tests useful in the clinic as well as in the laboratory.

The individuals responsible for this volume serve without stipend and are indeed deserving of the appreciation of all who are concerned with the preparation, prescription, and use of drugs.

JOSEPH M. WHITE

FACTORS REGULATING BLOOD PRESSURE. By B. W. Zweifach and Ephraim Shoor, Ed. New York, Josiah Macy, Jr., Foundation, 1949. 280 pp. $2.55.

This book is the report of the transactions of the third conference of this group. At the first and second conferences previous progress in the field of hypertension and vascular disease was evaluated. The third conference is concerned with specific problems in hemodynamics, the original goal of the group. The members have discussed the hemodynamics of venous, capillary, renal circulation; the measurement of blood pressure by (1) capacitance manometer and (2) differential impedance recorder; and the circulatory factors in cardiovascular stress and hypertension.

In contrast with the previous conferences all speakers, except Dr. Benjamin Zweifach, were guests of the group. These guests were: H. C. Bazett, Louis N. Katz, Harold Lamport, F. Noble, W. C. Ballard, Jr., Eugene A. Stead, Andre Cournand, K. S. Grimson, and Seymour S. Kety. In addition, several other guests were present and participated in the discussion.

The informal nature of the conference groups has been maintained, and many valuable remarks from the floor have been presented. The papers cover in detail much of the work of the speakers, and will be invaluable to readers doing advanced research on cardiovascular diseases and hemodynamics. However, there is also included a great deal of subject matter which will enlighten and interest the reader who has only an acquaintance with the cardiovascular system.

J. F. S.

NURSING IN THE PREVENTION AND CONTROL OF TUBERCULOSIS. By H. W. Hetherington and F. W. Eshleman. New York, G. P. Putnam's Sons, 1950. xvi + 361 pp. $4.50.

In these times of increased emphasis on tuberculosis in the curriculum of schools of nursing, both students and instructors welcome the publication of a good text on the subject. This book was first published in 1941, was revised in 1945 and again in 1950. The latest information regarding such medical topics as antibiotics and the controversial subjects such as BCG are therefore included.

A survey of the table of contents reveals the extent of the material covered, from an introductory consideration of pulmonary tuberculosis itself—its epidemiology, etiology, pathology, symptomatology, diagnosis and treatment—to the public health aspects of tuberculosis, specifically, prevention, case-finding, a consideration of the chest clinic and the family service, ending with family case studies and a short chapter on rehabilitation. Between these two broad sections are included chapters on nursing responsibilities in patient management, tuberculosis and pregnancy, tuberculosis in infants and children, and the prevention of tuberculosis among nurses.

As may be seen from this brief survey of contents, the scope of the book is wide. Its emphasis is two-fold. It seeks to give the nurse an understanding of the clinical and therapeutic aspects of the disease through such meticulously written chapters as that on diagnosis with its detailed explanation of examination, x-ray, and laboratory studies. Having achieved this, it considers the wide implication of tuberculosis prevention and control, and the nurse's role in such a program.

Considering this book as a text in the hands of students and instructors, the following assets are noted: distinct topical headings, an excellent selection of up-to-date references at the end of each chapter, and an appendix of guide outlines for the presentation of family history, medical findings, and patient history at clinical or ward conferences.

It is only in the general organization of its material that one might take objection to this text. It is not presented in the most logical sequence and is therefore not clear in outline. By the same token, certain topics are subject to repetition; as for example, the regimen of the tuberculosis patient, which is treated at length both in Chapter III and Chapter X; whereas other topics, such as extrapulmonary tuberculosis, the surgical treatment of pulmonary tuberculosis and its surgical nursing care, are rather too sketchily treated. This reservation notwithstanding, *Nursing in Prevention and Control of Tuberculosis,* is highly recommended to the attention of all those interested in this field. It fills a very serious need in a clinical area which has as yet produced few textbooks.

SUE CAILLIET

SURGICAL NURSING. By Eldridge L. Eliason, L. Kraeer Ferguson, and Lillian A. Sholtis. 9th ed. Philadelphia, J. B. Lippincott Co., 1950. xxv + 728 pp. $4.00.

This revision of *Surgical Nursing* clearly shows the changing trend in thought in the presentation and teaching of this subject. The emphasis is on the patient and how he, as an individual, may be helped best before, during, and after surgery so that he can resume his normal activities after discharge from the hospital.

The surgery of each system is discussed in detail and there are excellent bibliographies after each chapter listing timely articles from current publications. Anatomical discussions, though brief, are clear and concise with many new drawings and diagrams. The greatest stress is laid on the nursing care of the patient with practical suggestions as to the teaching which can be done and to the role of the nurse in the rehabilitation of the patient. Questions in the form of a specific clinical situation emphasize this.

The authors have included current operative techniques and the uses of the newest antibiotics. They have added excellent chapters on the most recent advances in thoracic, cardiac, and neurosurgery.

ROSALIND LOUTREL

Yale Alumni in Medicine

ALUMNI DAY REMARKS

February 22, 1950

ELIHU S. WING, President, Yale Alumni in Medicine

It is my privilege as president of the Yale Alumni in Medicine to extend greetings to all of you on this, Our Day, at our medical alma mater. On behalf of the alumni, I assure you—members of the Executive Committee of the Alumni in Medicine, and you, the administrative and faculty members of Yale University School of Medicine—of our sincere appreciation of your fine cordiality and of your excellent program of scientific and social activities. We all agree that it exceeds in scope and interest the Alumni Day programs of the past. We are given the opportunity to see the school in operation; to observe its teaching; to witness its exhibits, its functioning laboratories, and its vast research projects; to admire its fine libraries; and to attend grand rounds, symposiums, and conferences of the most interesting and instructive kind. We thank you most heartily for all that you have done for us.

Of course, what most impresses many of us today is the great change over the years: old landmarks are gone, and old familiar faces. Such change is typical of the American way of growth in an age of rapid scientific progress, when the field of medical knowledge and scientific activity has so widened that new problems have and are continually confronting the school. Today, it is a great satisfaction to know, that, in keeping with the improvement in its physical equipment, the school will not only continue to emphasize but add emphasis to its fundamental function, namely, adequate and high-grade teaching. In the old days, we had superb teaching by inspiring leaders, but poor and meager equipment and little research work. Today, we have excellent equipment (though needing further modernization) developed by the efforts of recognized leaders in medical science—to whom we are glad to pay tribute.

Now, there is a phase of the school's activities that is not so obvious as we look around within its walls. I refer to its interest in carrying extramural postgraduate medical instruction to outlying areas of the state, in co-operating with many hospitals, with the State Medical Society, and with other health-promoting agencies, thus taking on the functions of a medical center. Dean Long, I am sure, will elaborate upon this important contribution to medical practice. I trust that all of you have read his excellent 1948-49 report as Dean, and President Seymour's recent favorable comments upon it. This significant expansion of our medical school's extramural influence represents an important trend in a changing pattern of postgraduate education and of medical care.

For years, the concept of a co-operative approach to medical care has

had an able and influential advocate in Dr. Alan Gregg, Director of Medical Sciences of the Rockefeller Foundation. Let me quote a sentence from an address of his on "The Future of the Voluntary Hospital" delivered nearly six years ago at the centennial celebration of Butler Hospital: "Institutions must change to meet . . . their outside relations, if they are to keep significant." That is as true of the voluntary medical school as of the voluntary hospital, and I may add that the best type of voluntary hospital is the one associated in some way with a medical school. It is only just to acknowledge that for years there have been instances and places where some form of cooperative medical care has been successfully carried on—so the idea is not new. But now the most encouraging feature of it all is that not only is the idea becoming more widespread, but that most practising physicians are eager to avail themselves of this postgraduate instruction and thereby to improve their ability to better serve their patients. Whatever may come later in the way of regimentation in our medical affairs, this form of evolution in postgraduate education and in medical care is sure to continue, and we should be proud that Yale is so whole-heartedly behind such a program. The keen interest of the Corporation and President of Yale University and their support of medical affairs at Yale is most heartening.

In concluding, we, the alumni, must remain conscious of Yale's considerable initial investment in us, of its growing assistance in keeping us professionally fit; and, parenthetically, I wonder if we all realize that the alumni of no other single department of the many departments of Yale University are as dependent upon their department in after years, as are we, the graduates of medicine. I do not wish to take up more valuable time as there are others on the program whom we are all anxious to hear since they have messages relating to the future of this school, its policy and future programs, as well as how we alumni fit into the picture. I wish to leave with you, however, this challenge. After today's re-acquaintance with the school's varied activities, after learning more about its growing usefulness and influence, and being conscious of its desire to extend and make its teaching program second to none, let us be sure that we not fail to assume our proper and fair share of responsibility in the venture.

A PERSONAL ACCOUNT OF THE ASSOCIATION'S MEETING ON SUNDAY AFTERNOON, JUNE 11, 1950

MIRIAM K. DASEY

A cool, delightful summer day, the thermometer steady and no change in weather forecast: for me it was my most important day at Yale. It began mid-morning with the delivery of an orchid; a purple bloom with white petals and festive loops of white ribbon. Then came word from Ethel Kushlan that there was a very good "write-up" in the New Haven Register, and that she was sending the children among her neighbors asking them to save that section for her so that she could send it to me, knowing that members of my family and friends would be interested. All good omens were leading up to three-thirty when the Kushlans called for me.

At four the Association of Alumni in Medicine was ready to convene, and with Sam as my sponsor I was led to the front of the Historical Library, facing the audience, to occupy one of the long seats with Dr. Grover F. Powers and Dr. Samuel D. Kushlan. Dr. Winternitz sat on our left; Dean Long, Dr. Clyde Deming, Dr. Levin Waters, and Dr. Roswell Gallagher sat opposite us. This was an annual meeting, and a few business items were presented. Then the presiding Second Vice-President, Dr. Gallagher stepped back and Dr. Waters took over. With anecdotes and light persiflage, but with a strong undercurrent of seriousness and affection, he presented to Dr. Winternitz the Milton C. Winternitz Number of the *Yale Journal of Biology and Medicine*—containing articles written in honor of Dr. Winternitz by his former students, assistants, and friends. Dr. Winternitz uncurled himself from the capacious leather chair which he had occupied so casually and rose. He thanked Dr. Waters, and through him the many friends who had contributed to *his Journal;* and then, in recognition of his long friendship with Dr. George H. Smith, the founder of the *Yale Journal of Biology and Medicine,* he launched into an eulogy of what Dr. Smith had accomplished. Dr. Winternitz's speech became the climax to the successful career of the first editor of the *Journal,* Dr. Smith.

The next number on the program was the presentation of the Miriam Kathleen Dasey Award. Dr. Grover F. Powers, Chairman of the Committee, whose support and interest has been its strong recommendation among the alumni and faculty, rose to explain what it was all about. He said:

> Twenty-nine years, three months, twenty-four days! Such is the cold mathematical chronicle of the professional career at Yale of the lady who as Registrar of the School of Medicine retires in nineteen days days to the Hub, there to keep an ever-watchful eye, we hope, on Yale's traditional rival! In the lifetime of an individual, a generous span of years! In the history of this School, a dynamic period of great achievement! In personal accomplishment amidst challenging problems, fruitful years in enduring values written into the lives of hundreds of students and faculty alike, and into constructive policies of this School!
>
> It is fitting that we of alumni and faculty express our gratitude for a life so lived in a memorial of continuing tribute through the years. With funds at hand (approximately $3,000*), and those anticipated in the future, Yale University is asked to establish the Miriam Kathleen Dasey Award in the School of Medicine. Dependent upon the resources available at the time a presentation is under consideration, and upon the judgment of proper authorities, the Award may take the form of a loan, a scholarship, or a prize to a member of the student body. Miss Dasey desires that the recipient of the Award be a student who seems destined to fulfill the characterization of a beloved physician as expressed by Dr. Francis W. Peabody, in these words:
>
> > "The good physician knows his patients through and through, and his knowledge is bought dearly. Time, sympathy, and understanding must be lavishly dispensed; but the reward is to be found in that personal bond which forms the greatest satisfaction of the practice of medicine. One of the essential qualities of the clinician is interest in humanity; for the secret of the care of the patient is in caring for the patient."

*$3,426 as of January 11, 1951.

It is hoped that this concept is embodied in the citation which will be presented to Miss Dasey. It reads as follows:

In recognition of over twenty-nine years
(March 1921-July 1950)
of devoted and distinguished service as
REGISTRAR
Alumni and Faculty
of
Yale University School of Medicine
have established the
MIRIAM KATHLEEN DASEY AWARD
To be presented annually (or as otherwise determined) to that student who by strength of character, personal integrity and academic achievement gives promise of fulfilling the ideal of the compassionate physician.
June eleventh
1950

It is now my pleasure to ask the treasurer of the Miriam Kathleen Dasey Fund, Dr. Samuel D. Kushlan, class of 1935, to present to Miss Dasey a volume of letters of affection and appreciation which he has received for her. And also the citation appropriately inscribed by our Medical Artist, Mr. Armin Hemberger.

Once or twice, *sotto voce,* I asked Sam Kushlan if I had to do anything. Being a fine physician and a fine psychologist he was very reassuring: I had no responsibilities. The result was that I was completely relaxed by the time Dr. Winternitz finished speaking. Then the question came to me: If he commented upon his honor, should I do so too? Again I had recourse to Sam. And then in the tone which he might have used to his young son David, he said, "Well, you could thank them, perhaps; and you could tell them you are going to live in Brookline." By this time Dr. Powers had finished his fine presentation of the Award, saying that Dr. Kushlan would carry on from there. With two flat, square, festive packages wrapped in white paper and blue ribbon in one hand, and with the other under my elbow, he urged me from the depths of my comfortable seat, led me to the lectern, gave me the packages, and with a beaming smile at the audience and at me, he left me—on my own. As I recalled all that the Miriam Kathleen Dasey Award represents for the future, its expression of friendliness of the past, and the friendship of Dr. Powers and Dr. Kushlan, I found that it was easy to express myself. I prefaced my remarks by reporting my *sotto voce* conversation with Sam. I told them that I did thank them for their friendliness and loyalty. I told them that I had found an apartment in Brookline at 374 Chestnut Hill Avenue and that I should always be glad to see any Yale man. I told them that my work at the School of Medicine

had always been interesting and stimulating; that during the war and postwar period it had been predominantly concerned with admissions which I regarded as its most important function in its relation to the University. There was always the excitement of accepting an outstanding student which offset the routine work involved in screening those less desirable. However, sometimes these objects of our enthusiasm were also interested in Harvard, and their statement that they had been accepted by Yale was sufficient to gain admission to our rival institution. Of course it was highest recognition of the screening ability of our Committee on Admissions; but it rankled. Having lived in the Near East I knew about the Evil Eye and its potentialities, so next October, when the weather was cool and delightful and most of my friends were indoors working, I planned to stroll through Longwood Avenue, and cast the Evil Eye on the Harvard Medical School. I was sure that almost immediately thereafter the tables would be turned; there would be an influx of candidates who had been accepted by Harvard but preferred to study at Yale. Or lacking this, there might be an epidemic among the Harvard personnel, students, and faculty. Thus I would feel that I had done one more thing for Yale. I then retreated (I hoped not in confusion) with my honors modestly masked by their gay coverings.

Dean Long then ended the session by thanking the Yale Alumni in Medicine for their interest and co-operation, commenting on the value of the Association to the School, and of its grateful recognition by the School. There followed a social hour in the Lounge of the Sterling Hall of Medicine—cocktails, tea, and delicious sandwiches, all served with warm hospitality.

And so ended my day. I really felt that my career at Yale had ended at this point; it was one of fulfillment.

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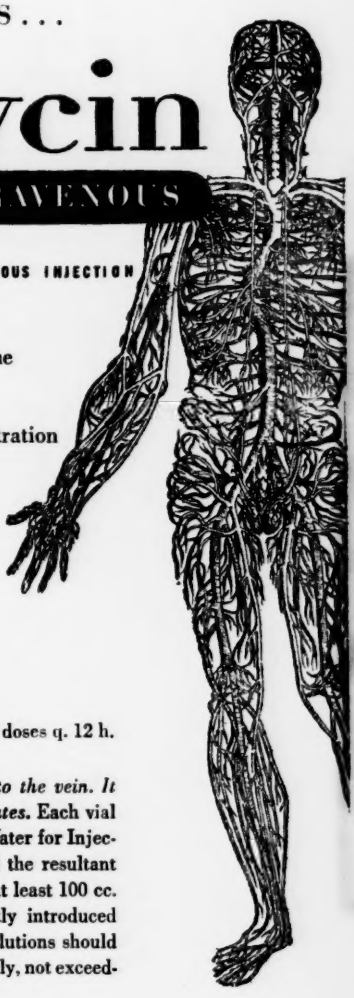

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